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CEREAL CHEMISTRY

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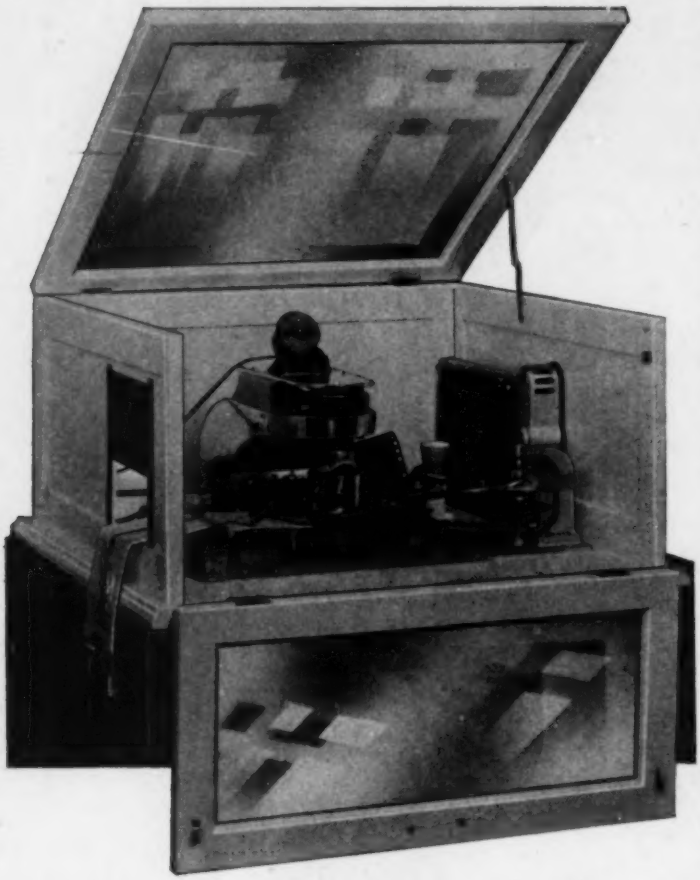

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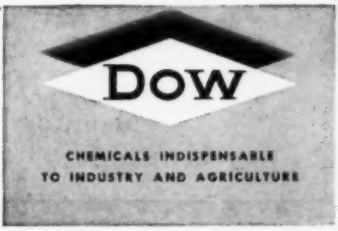

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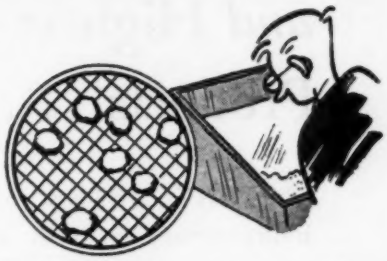

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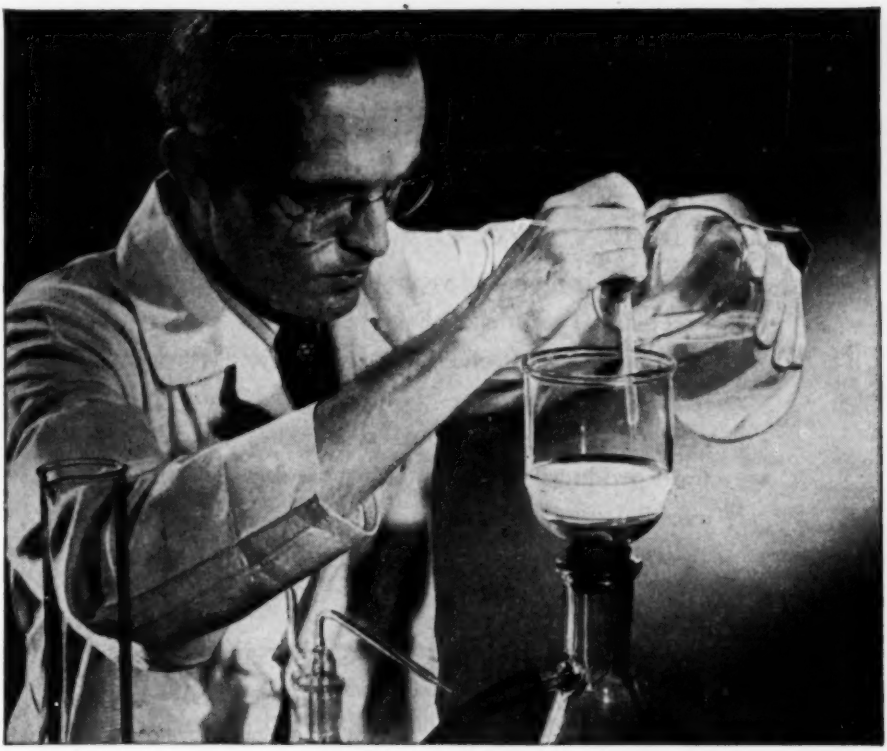

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CEREAL CHEMISTRY

VOL. XXV JULY, 1948 NO. 4

A COMPARATIVE STUDY OF SOME PROTEIN FRACTIONS OF WHEAT FLOUR [1]

W. DERBY LAWS [2] and W. G. FRANCE [3]

ABSTRACT

An electrophoresis investigation of some protein fractions of flour from various wheats was carried out with the idea that it might reveal the presence of different protein components in the gluten complex. It proved impractical to study the proteins in either alcoholic buffers or in aqueous alkaline buffers with a pH of 10.2. Therefore, attention was turned to the electrophoretic study of gluten proteins dissolved in acetic acid and dialyzed against citric acid–disodium phosphate buffer, pH 2.15. However, this procedure failed to reveal any significant difference in the flour proteins. Electrophoresis of the water extract of Chiefkan, Early Blackhull, and Comanche flours gave similar electrophoretic patterns for all flours.

Baking tests with flour fortified with wet and dry gluten washed from the various flours appear to confirm the results obtained by electrophoresis technique, namely, that the glutens from the flours studied are very similar in properties. When flour blends were fortified with wet, freshly washed gluten, the Chiefkan gluten gave the greatest increase in loaf volume and the Comanche the least for a given amount of protein added.

This work was one phase of an investigation of the factors and constituents in wheat which are responsible for quality in flour. The general plan of the investigation was to compare the characteristics of poor quality flour *vs.* good quality flour. Chiefkan and Red Chief wheats were selected as varieties which generally give poor quality flour, Pawnee and Comanche wheats as the varieties which generally yield good quality flour, and Early Blackhull and Wichita wheats as the varieties which are probably intermediate between the other two groups. In order to make the samples comparable they were collected in triplicates, one member of each type from a given location, and each set of three collected from a different location so that samples were

[1] Manuscript received February 16, 1948.
Contribution from the Ohio State University Chemistry Department; represents a portion of the work carried out under a cooperative program by Pillsbury Mills, Inc., and the Ohio State University Research Foundation.

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[3] Professor, Department of Chemistry; deceased December 4, 1947.

obtained from three widely separated areas. The samples collected, the location from which obtained, and the baking quality of each are presented in Table I.

Cereal chemists generally agree that the properties of wheat gluten determine flour quality. Consequently, many attempts have been made to find measurable differences in the protein fractions which could be shown to be responsible for the difference in the quality of the flour. An electrophoretic investigation of some protein fractions of the flour was undertaken with the idea that it might reveal the presence of different protein components in the flour from the three groups of wheat being studied.

TABLE I

WHEAT SAMPLES STUDIED

Variety	Location	Designation	Baking quality
Chiefkan	Protection, Kansas	Cp	Very poor
Early Blackhull	Protection, Kansas	Bp	Fair
Comanche	Protection, Kansas	Hp	Fair
Chiefkan	Jetmore, Kansas	Cj	Very poor
Early Blackhull	Jetmore, Kansas	Bj	Poor
Comanche	Jetmore, Kansas	Hj	Poor
Red Chief	Newton, Kansas	Rn	Very poor
Wichita	Newton, Kansas	Wn	Fair
Pawnee	Newton, Kansas	Pn	Poor
Early Triumph	Caldwell, Kansas	Ec	Good

The electrophoresis apparatus suitable for the quantitative study of proteins by the moving boundary method has been much improved and widely used until it has become one of the most powerful tools available to biological chemists for the study of protein systems. The moving boundary electrophoresis method is applicable to a wide variety of high molecular weight substances, both in their native and denatured form, and yields information as to the number of electrically separable components present in the mixture and the electrical homogeneity, concentration, and mobility of each component. Since this method is adapted to the study of a wide variety of proteins and protein mixtures in dilute aqueous buffers, it seemed to offer the best means of detecting differences in the proteins of good quality and poor quality flours despite the lack of suitable neutral solvents for gluten proteins.

The present investigation was not an attempt to determine the definite composition of the gluten proteins from the various flours but rather to make a comparative study of the glutens from each flour.

Experimental

No attempt was made to separate the gluten into fractions for this study. Instead, either the whole gluten mass or protein extracted directly from the flour was used. A Tiselius electrophoresis apparatus manufactured by the Klett Manufacturing Company was used for the electrophoretic determinations.

Electrophoretic determinations were made on the protein fractions of flour from each series of wheat samples presented in Table I.

The preliminary determinations were made using alcoholic buffers which contained 50% of ethyl alcohol by volume. These buffers were

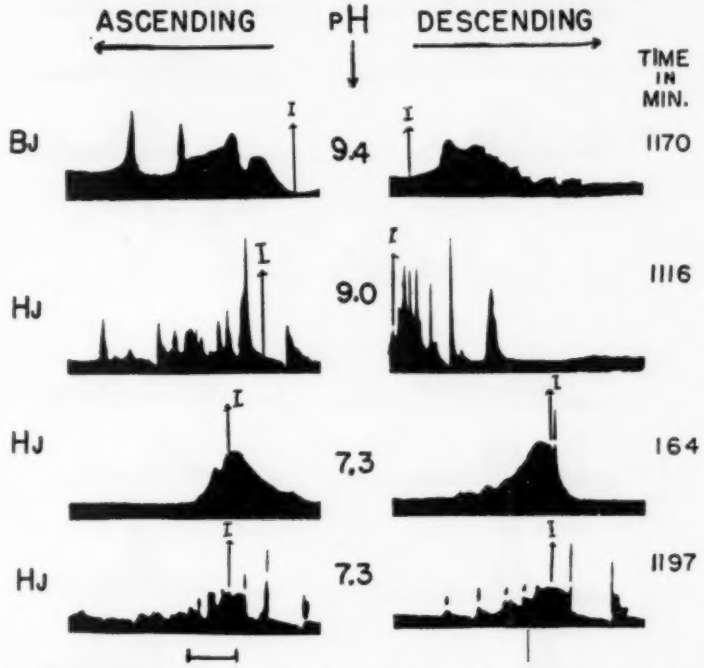

Fig. 1. Electrophoresis patterns of alcohol-soluble flour proteins in 50% alcoholic buffers. (Bj field strength 8.0 volts/cm.; Hj (pH 9.0) 9.2 volts/cm.; Hj (pH 7.3) 8.3 volts/cm. "I" indicates initial boundary. Line equals 1 cm. Descending boundary at negative (−) pole.)

prepared by diluting aqueous buffers prepared after the manner of Clark and Lubs with sufficient 95% alcohol to give a solution of desired alcoholic concentration. An aqueous buffer of pH 8.0, when thus diluted, resulted in a buffer of pH 9.0–9.4, and one of pH 6.0 gave an alcoholic buffer of pH 7.3.

The method of making all the electrophoretic determinations was essentially as described in the literature (1) (6). Runs were made at a temperature of 2.5°C. As a general rule it required from 10 to 20 hours at a field strength of about 8.5 volts per cm. to complete a de-

termination in alcoholic buffers. Although patterns were obtained (Fig. 1) no mobilities were calculated because, in addition to the difficulty of determining the pH and ionic strength of the buffer in alcoholic medium, the long periods of electrophoresis and the enforced study at a temperature above that of maximum density of alcohol-water mixtures result in a marked increase in boundary disturbances due to thermal convection (8).[4]

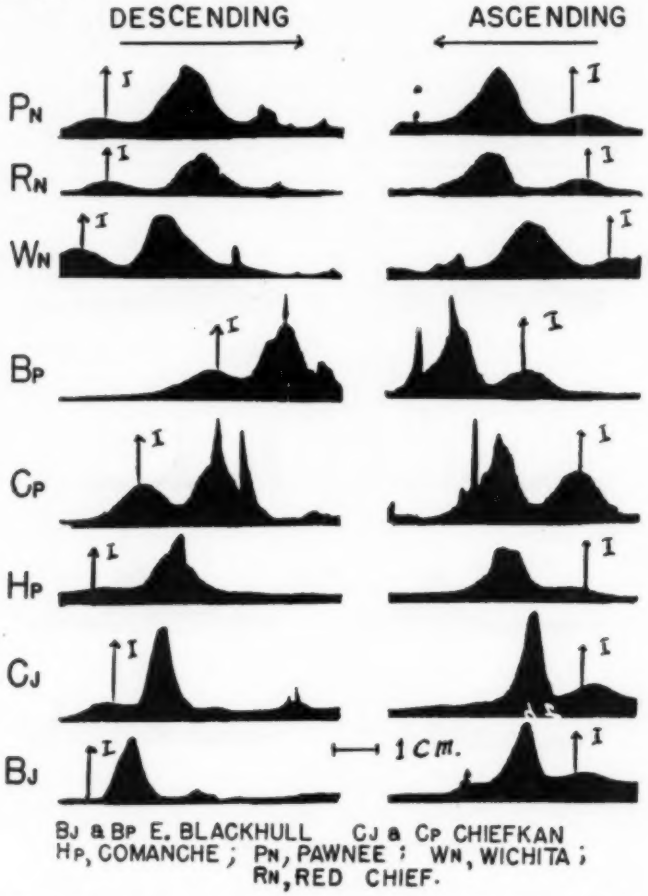

Fig. 2. Electrophoresis patterns of gluten proteins from Hard Red Winter wheats. (Boric acid-sodium hydroxide buffer pH 10.2. X = field strength, T = time in minutes. For Pn, X = 3.87, T = 269; Rn, X = 3.99, T = 258; Wn, X = 3.87, T = 262; Bp, X = 4.06, T = 205; Cp, X = 4.17, T = 205; Hp, X = 4.09, T = 214; Cj, X = 3.61, T = 198; Bj, X = 3.51, T = 203. "I" indicates the initial boundary. Line equals 1 cm. Descending boundary at negative (−) pole.)

Since the above procedure was not satisfactory, attention was turned to electrophoretic studies in aqueous buffers. Cook and Rose (3) (4) have shown that gluten can be almost completely dissolved

[4] The above paper was published after our work with alcoholic buffers had been discontinued in January, 1947.

in 10% sodium salicylate without undergoing hydrolytic changes on long standing. Accordingly, a 10% solution of sodium salicylate was selected as a solvent. A sodium hydroxide–boric acid buffer with a pH 10.2 (original ionic strength, 0.094) prepared after the manner of Clark and Lubs was used. The flour samples from the three locations were studied and the patterns obtained are presented in Fig. 2.

Although it is well known that gluten proteins undergo irreversible denaturation in alkaline solution, it seemed probable that any marked

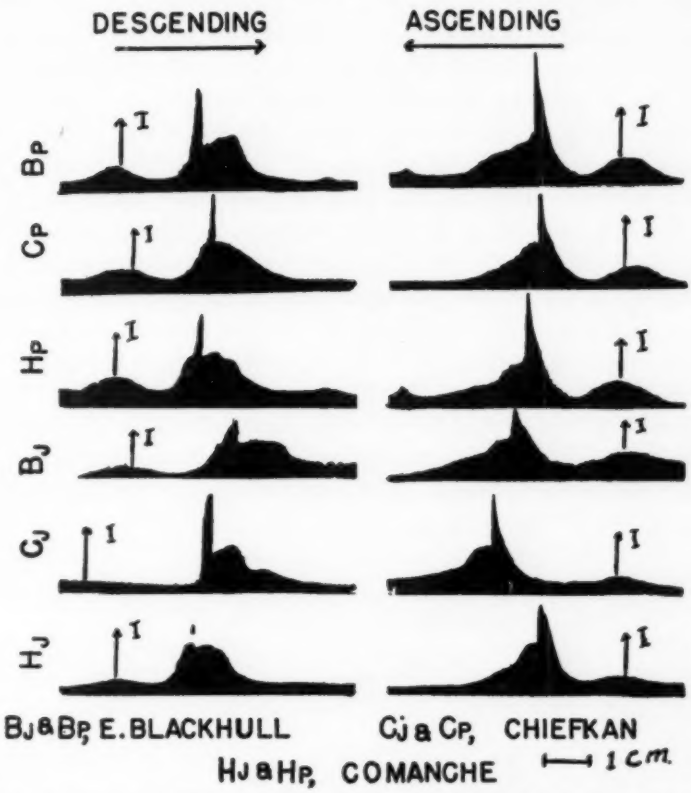

Fig. 3. Electrophoresis patterns of gluten proteins from Hard Red Winter wheats. (Boric acid-sodium hydroxide buffer pH 10.2. Ionic strength 0.047. Field strength × time equals 67,500 volt-sec./cm. for Cp, Hp, and Bp; 91,560 for Bj; 96,620 for Cj; and 59,320 for Hj. "I" indicates initial boundary. Line equals 1 cm. Descending boundary at negative (−) pole.)

difference in the protein composition of the gluten from the various flours would appear in the electrophoresis patterns as additional components even though the proteins were denatured. Since this investigation did not try to characterize the various components of the gluten proteins but merely attempted to compare the number of electrically separable components present in the glutens of good quality and poor quality flour, it would seem to matter little whether the protein com-

ponents were denatured or not so long as they remained electrically separable. (For present purposes it was necessary to assume that they do.)

The ionic strength of the buffer was about twice as high as considered necessary for protein solutions of the concentration used, which varied from 0.3% to 0.4%. Therefore, a study was made of gluten from some of the same samples, using the same buffer with the ionic strength reduced to 50% of the original value. The diagrams obtained are presented in Fig. 3.

In both cases the samples were prepared by stirring 7.5 g. of wet, freshly washed gluten in 250 ml. of 10% sodium salicylate, in a Waring Blendor, for 5 minutes, centrifuging for 10 minutes at a centrifugal acceleration 400 times gravity to destroy the foam and remove traces of starch, and dialyzing 100 ml. for 48 hours against 2 liters of the respective buffers. Dialysis was carried out in the cold room at 2°C. The buffer was not changed during dialysis but the protein solution was in equilibrium with the buffer in every case as shown by the measurement of the specific conductance of each at the beginning of the run. Just before placing in the electrophoresis cell the sample was again centrifuged for 10 minutes, in the cold room, to remove the material which salted out.

It should be noted that in all these electrophoretic determinations only about 40% of the gluten added remained in suspension after dialysis. The data in Table II show the amount for the individual samples.

TABLE II

PORTION OF ADDED GLUTEN REMAINING IN SUSPENSION AFTER DIALYSIS AGAINST A SODIUM HYDROXIDE–BORIC ACID BUFFER, pH 10.2

Variety	From Jetmore	From Protection	Variety	From Newton
	%	%		%
Chiefkan	45.7	37.2	Red Chief	44.1
Early Blackhull	42.5	40.5	Wichita	45.6
Comanche	36.7	35.7	Pawnee	39.0

Since it was not possible to control the composition of the sample, making it necessary to assume that the same protein fraction remained in suspension for each sample and on each duplicate run, a study of various buffer systems was made and it was found that a citric acid–disodium phosphate buffer was more satisfactory because a greater portion of the added gluten remained in suspension after dialysis. The data in Table III show the portion of gluten in the original acetic acid suspension remaining in solution after 48 hours dialysis.

The procedure used in preparing the sample was as follows: 7.5 g. of freshly washed, wet gluten were suspended in 250 ml. of 0.07 *N* acetic acid by stirring for 5 minutes in a Waring Blendor. The suspension was then centrifuged for 10 minutes at an acceleration 400 times gravity to remove traces of starch and destroy the foam formed

TABLE III

PORTION OF ADDED GLUTEN REMAINING IN SUSPENSION AFTER DIALYSIS AGAINST CITRIC ACID–DISODIUM PHOSPHATE BUFFER, pH 2.15

Sample	% Protein for gluten	% Protein for flour
Chiefkan (Cj)	88.6	91.2
Early Blackhull (Bj)	93.7	—
Comanche (Hj)	96.1	92.0
Early Triumph (Ec)	93.4	98.3

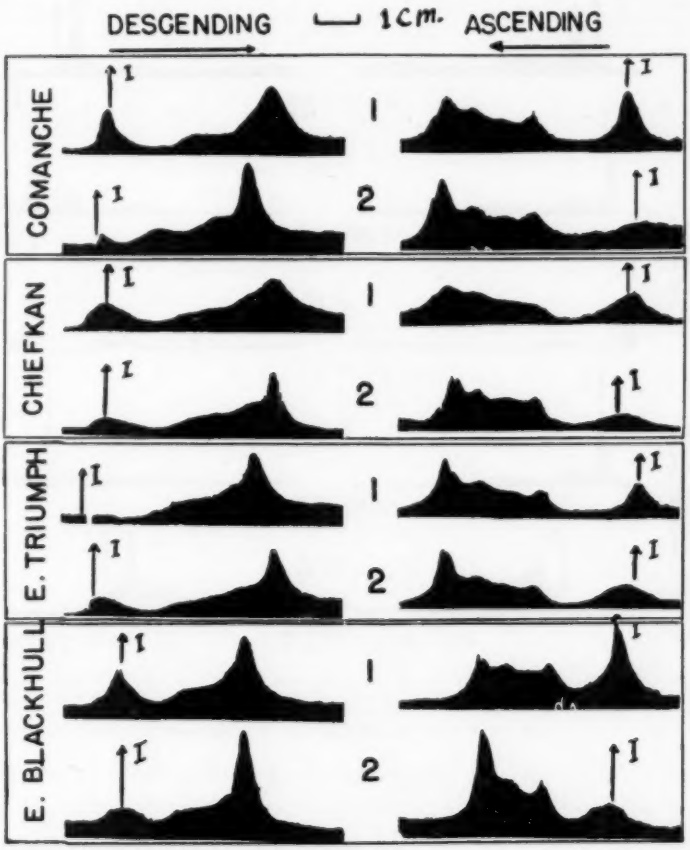

Fig. 4. Electrophoresis patterns of Hard Red Winter wheat protein. Picture No. 1 for protein extracted directly from flour and No. 2 for dissolved gluten. (Citric acid-disodium phosphate buffer, pH 2.15 and ionic strength 0.03. Field strength × time = 85,320 volt-sec./cm. for Comanche, Chiefkan, and Early Triumph and 66,400 volt-sec./cm. for Early Blackhull. "I" indicates initial boundary. Line equals 1 cm. Descending boundary at positive (+) pole.)

during stirring. Of the suspension thus obtained 100 ml. were then heated to 92°C. and held there for 2 minutes to destroy the proteolytic enzymes present. It has been shown that this treatment does not denature the protein (7). After heating, the sample was diluted sufficiently with buffer to give a solution of 0.26% protein. It (100 ml.) was then dialyzed against 2 liters of a citric acid–disodium phosphate

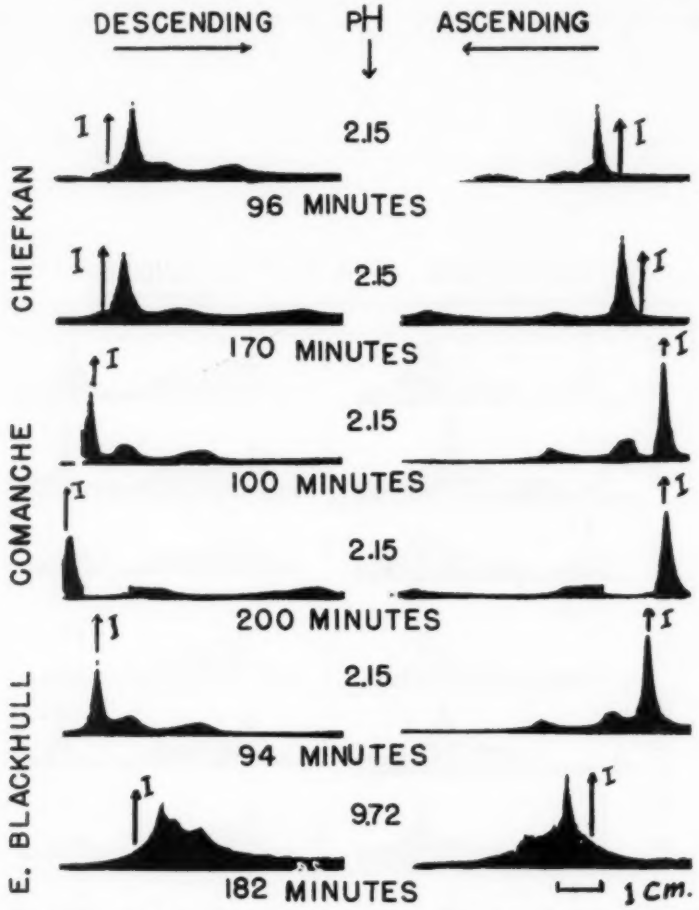

Fig. 5. Electrophoresis patterns of water-extractable proteins from Hard Red Winter wheat flour. (Field strength for Chiefkan 4.21, Comanche 4.24, E. Blackhull (pH 2.15) 4.20, and (pH 9.72) 4.26 volts/cm. Ionic strength 0.03. "I" indicates initial boundary. Line equals 1 cm. Descending boundary at positive (+) pole. For E. Blackhull, pH 9.72, descending boundary at negative (−) pole.

buffer of pH 2.15 and an ionic strength of 0.03 in the cold room (2°C.) until equilibrium between buffer and sol was reached as shown by conductivity measurements (72 hours), and centrifuged again just before placing in the electrophoresis cell. The patterns obtained are presented in Fig. 4. The top pattern, No. 1, of each pair in Fig. 4 was made of protein extracted directly from the flour. In this case

20 g. of flour were stirred in 250 ml. of 0.07 *N* acetic acid; otherwise the procedure used in preparing the samples was exactly as described above. The time for all runs was such that the product of the field strength and the time was a constant, as suggested by Schwert *et al.* (9). The system was tested for electrical leaks at the beginning and end of each run. At the conclusion of a run the current was reversed and the movement of the components observed 1½ hours. Any leak in the cell causing backward movement of the boundary would be greatly amplified by this procedure and thus readily detected.

The work of Finney (5) and also observations made in this laboratory indicate that the water-soluble protein may be more important to flour quality than has formerly been supposed. Therefore, an electrophoresis study was made of *distilled water extracts of the flour* using the critic acid–disodium phosphate buffer described above. The patterns obtained are presented in Fig. 5. All flour samples gave patterns that are alike and that have the same number of components. The Early Blackhull sample was also run at pH 9.72 and gave an electrophoresis pattern entirely different from that obtained for the same sample at pH 2.15.

Discussion

The patterns shown in Fig. 3 are essentially the same for all six flour samples studied and there was little similarity between patterns of proteins from the same flour sample run at different ionic strength (see Fig. 2 and Fig. 3). The higher ionic strength appears to be best suited to a study of this type since there is evidence of better component separation in the patterns in Fig. 2 than in Fig. 3.

There was no essential difference in the patterns obtained from the various flour samples when an acidic buffer, pH 2.15, was used. The descending and ascending boundaries (Fig. 4) were not symmetrical, indicating component interaction. The patterns for protein extracted directly from the flour and for gluten were very nearly identical except for the slow-moving component which was much more prominent on the patterns made from the protein extracted directly from the flour. This was probably due to the presence of part of the water-soluble fraction in the suspension, as it was shown that the main component from the water-soluble protein has a very low mobility also. There would be little water-soluble protein present in the gluten suspension because of the method of sample preparation.

The slow-moving "component" moved so very little that it could easily be taken for the well-known delta and epsilon anomalous boundaries except for the fact that the area under the remainder of the peaks (for the patterns of protein extracted directly from flour, No. 1, Fig. 4)

only accounts for about 80% of the protein present in the solution, and the area of the first peak must be taken into consideration in order to account for all the protein present. This is shown clearly in Table IV. These data indicate that the first peak in these patterns represents a true component.

TABLE IV

CONCENTRATION OF THE VARIOUS PROTEIN SOLUTIONS REPORTED IN FIG. 4

Flour sample	Protein concentration of solutions			
	First peak	Other peaks	Total	By Kjeldahl
	%	%	%	%
Comanche	0.05	0.18	0.23	0.26
Chiefkan	0.05	0.20	0.25	0.25
E. Triumph	0.04	0.18	0.22	0.26
E. Blackhull	0.06	0.16	0.22	0.25

The electrophoresis patterns of the water-soluble protein of flour presented in Fig. 5 show that the patterns for all flours were alike. There was one main component of very low mobility (see Table V) and two minor components which had a higher mobility than the

TABLE V

ELECTROPHORETIC MOBILITIES OF PROTEIN COMPONENTS IN WATER EXTRACTS OF VARIOUS FLOURS, pH 2.15 (CM./SEC./VOLTS/CM. $\times 10^5$)

Chiefkan (Cj)		Early Blackhull (Bj)		Comanche (Hj)	
Ascend. boundary	Descend. boundary	Ascend. boundary	Descend. boundary	Ascend. boundary	Descend. boundary
0.69	0.67	0.03	0.06	0.01	0.07
4.06	3.58	3.14	2.97	2.34	2.19
10.35	10.38	9.84	9.46	9.81	9.42

major component. The mobilities of the protein components from Chiefkan flour were slightly higher than those for the other two samples. The outstanding difference was for the main component. There was a tendency for the slower moving of the two minor components of the Comanche sample to separate into two peaks; otherwise, the patterns for water-soluble protein did not vary from one flour sample to another. The patterns for each boundary are symmetrical for all determinations made at pH 2.15. However, when Early Blackhull was run at pH 9.72 the boundaries were not symmetrical, indicating a need for a more detailed study of the various protein fractions in buffers of several pH's.

Schwert, Putnam, and Briggs (9) give a concise discussion of component interaction in electrophoretic work and point out that at least two types of interactions between protein components may be expected. When interaction between components occurs the patterns are not symmetrical with respect to the number or the relative areas under the peaks, and the mobilities of one or more peaks in each boundary will vary from that characteristic of any component. Certain types of interaction may be weakened by increasing the ionic strength of the buffer with the results that the patterns become more symmetrical.

There was little evidence of component interaction in the samples run at pH 10.2 and the higher ionic strength, since the patterns shown in Fig. 2 were nearly symmetrical in both legs of the cell. When the ion strength was reduced, the pattern became less symmetrical (Fig. 3), indicating a tendency toward component interaction.

When the electrophoretic analysis was made in acidic buffers of pH 2.15 and an ionic strength of 0.03 there was marked asymmetry in the two boundaries, indicating very decided component interaction. Since this investigation was a comparative study of the proteins of different varieties of wheat, no attempt was made to study the type of interaction taking place or to reduce the component interaction by changing the ion strength over this pH range.

The patterns presented in Fig. 4 show that the proteins from all flours studied apparently undergo the same type of interactions because all ascending boundaries are similar and all descending boundaries are similar.

Since the electrophoresis investigation failed to detect differences in glutens from Chiefkan, Early Blackhull, and Comanche flours, it was decided to test the "bread-improving" power of each of these glutens when added to a common flour. Three flour mixtures were prepared to be used as substrata to test the glutens. Mixture No. 1 contained 9.1% protein, No. 2 contained 11.1% protein, and No. 3 contained 13.1% protein, all on a 14% moisture basis.

Two samples of gluten were prepared from each of the Chiefkan, Early Blackhull, and Comanche samples from Protection. One was prepared by washing the gluten from the flour in 0.15% salt solution, drying and grinding to pass a 10XX bolting cloth. The other was prepared by purifying the gluten, as suggested by Baker *et al.* (2), before drying and grinding.

Each mixture was baked with given quantities of the finely ground gluten added. When the protein content of the mixture was increased 0.6% by adding dried gluten flours there was very little difference in the response of any mixture to the purified and unpurified glutens.

The Chiefkan gluten (Cp) gave the least improvement in the loaf volume and the Comanche (Hp) gluten gave the greatest improvement, although the difference in the two was probably not significant.

When the protein content of the mixture was increased by approximately 1.2% by the addition of dried purified finely ground gluten, the response of each mixture to the added gluten was in the same order as reported above. However, when the increase of 1.2% was made by adding freshly washed, unpurified wet gluten to the mixtures, the Chiefkan gluten produced the greatest response. Furthermore, the response to wet gluten was significantly higher (above 1% level) than the response to dry gluten even though the protein content was increased by the same amount in each instance. The results for wet and dried gluten are compared in Table VI.

TABLE VI

PROTEIN CONTENT (14% MOISTURE) AND LOAF VOLUME OF BREAD FROM FLOUR MIXTURES WITH ADDED GLUTEN

Sample	Gluten	Dry gluten		Wet gluten	
		% Protein	Loaf volume	% Protein	Loaf volume
Mixture 3	None	13.1	453	13.1	453
	Cp	14.5	573	14.2	654
	Bp	14.5	605	14.4	620
	Hp	14.4	587	14.4	634
Mixture 2	None	11.1	550	11.1	550
	Cp	12.7	609	12.7	615
	Bp	12.4	600	12.6	595
	Hp	12.4	621	12.8	617
Mixture 1	None	—	505	—	505
	Cp	—	523	—	641
	Bp	—	545	—	623
	Hp	—	550	—	615

In the light of the work reported here, which appears to agree with the work of Schwert *et al.* (9) who found no difference in the electrophoretic pattern of four gliadin samples regardless of the source of gliadin or method of preparation, it can be definitely stated that under the conditions of this investigation, the electrophoresis technique did not detect any significant differences in the proteins of the various flour samples. However, it should be emphasized that this does not mean it has been definitely established that differences do not exist. A more detailed study involving fractionation of the gluten into various protein fractions and improved solution techniques and buffer systems might detect small, but important, differences which were not

shown in the present investigation. However, the limited amount of electrophoresis data available on wheat proteins indicates that either there is virtually no difference in the composition of the gluten protein from poor quality and good quality flour, or the development and manipulation of the gluten during the sample preparation alters the protein in such a manner as to destroy any existing differences.

The baking data presented in Table VI appear to support this conclusion and indicate that the state of gluten development may be more responsible for flour quality than the chemical or amino acid composition of the gluten complex. This would agree completely with the electrophoretic work which failed to detect significant differences in the proteins from the various flour samples investigated.

Acknowledgment

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AIR, WATER VAPOR, AND CARBON DIOXIDE AS LEAVENING GASES IN CAKES MADE WITH DIFFERENT TYPES OF FATS [1]

MAUDE PYE HOOD [2] and BELLE LOWE [3]

ABSTRACT

The relative increase in cake volume over batter volume attributable to air, water vapor, and carbon dioxide was investigated. The major increase in cake volume was produced by carbon dioxide, followed by water vapor and air, in the order given. The effectiveness of water vapor in the presence of air in leavening cakes varied with the type of fat used, being greatest with oil, intermediate with butter, and least with hydrogenated lard. Conversely, the effectiveness of carbon dioxide was greatest in the hydrogenated lard and least in the oil cake.

Air-evacuated batters showed very little increase in cake volume, indicating that the effectiveness of water vapor as a leavening agent depended on the presence and distribution of air in the batter. The cakes from the air-evacuated batters were considered unpalatable, whereas those leavened by air and water vapor and by carbon dioxide, air, and water vapor were acceptable.

Viscosity of the batters was affected by the mobility of the fats used and by the incorporation of gas in the batter. It was not always a good criterion of cake quality.

This investigation was undertaken to determine the proportionate leavening attributable to air, water vapor, and carbon dioxide in plain cake. Three fats were used to study the relationship, if any, existing between the type of fat and the leavening power of the three gases.

Good aeration of cake batter has long been considered of paramount importance in the production of light, good-quality cakes. Dunn and White (4) have reported that steam cannot materially increase the volume of cake during baking unless air pockets are present into which the steam may vaporize. They estimated that approximately half of the increase in volume of pound cake was due to thermal expansion of air. In their calculation, however, the initial quantity of air incorporated in the batter was included in the volume from air expansion. When air in their cakes was completely exhausted the resulting batter was described as a "custard-like cream," and there was no volume increase during baking. They found that the occlusion of a very small amount of air in the batter resulted in a definite increase in cake volume. Results, when an evacuated batter was remixed to incorporate air, showed a reasonably good cake but with decided evidence of overmixing.

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Barmore (1) showed that of the 1,120- to 1,620-ml. increase in volume during baking of angel cakes, only 350 ml. could be attributed to the expansion of air, the remainder to steam. He postulated that the water vapor causing the expansion came from the sides and bottom of the cake. Hence, the steam that leavened the cake must pass through the interior of the cake to escape.

Carlin (2) observed that very few, if any, new gas cells were formed when baking powder was added to the cake formula. The carbon dioxide evolved seemed only to enlarge the air cells already present and not to form new cells. During baking the fat melted and the air spaces moved into the flour-water phase, apparently moving in a definite convection pattern until near the end of the baking when the movement was described as being "violent and without direction."

It was recognized that changes in type or proportion of ingredients, may necessitate changes in the method or extent of combining ingredients, to obtain a satisfactory cake. Hence, the method selected for combining ingredients was one, which over several years in this laboratory, had produced good quality cakes with the three types of fats selected for use in this study.

Materials and Methods

An oil,[4] butter, and a hydrogenated lard [5] were the fats selected for use. The butter was made by the Dairy Industry Department of the College from sweet cream. It had about 2.25% salt content with a current score of AA. It was used as obtained. A series of cakes (24 per series) was made with each fat. Each series of cake batters was further subdivided into three portions, in which different leavening agents were used, i.e., (1) air and water vapor, (2) carbon dioxide, air, and water vapor, and (3) water vapor alone.

The following formula was used:

Ingredients	*Grams*	*Grams per 100 g. of flour*
Fat	122	43.0
Salt	3	1.0
Sugar	150	52.0
Flour, cake	284	—
Milk, whole liquid	244	85.0
Egg-magma meringue:		
Whole liquid egg	96	33.8
Sugar	150	52.0

Baking powder (2.75 g./100 g. flour) was used in only one-third of the batter from each mix. A sulfate-phosphate type was chosen because it released only a small amount of carbon dioxide at room temperature.

[4] Wesson oil, The Southern Oil Company.
[5] Clix, Cudahy and Company.

All ingredients, except the milk and eggs, were incubated at 26°C. ± 1°C. Milk and egg were brought to 25°C. just prior to combining the ingredients. The work was conducted in a small laboratory in which the temperature was held at 25.3°C. ± 2.5°C.

Method of Combining Ingredients. The fat and salt were creamed on speed two of the Kitchen Aid mixer (Model G) for 30 seconds. Then 150 g. of sugar were added gradually during a 4-minute period. Creaming was continued for 10 minutes.

Approximately one-sixteenth of the flour was added to the creamed mixture with 20 strokes, using a hand balloon whip. About one-fifth of the milk was then added with 20 strokes. This procedure was repeated. Next one-half of the remaining flour and milk was added with 80 strokes. The remainder of the milk and flour was blended with the batter with 90 stirs of the hand whip.

The whole egg was beaten on speed three for approximately 1.5 minutes. Then the speed was reduced to two and the sugar added gradually while beating was continued for 4.5 minutes. The mixture was scraped down from the sides of the bowl, then beaten 30 seconds. The egg-magma meringue was folded into the batter with 25 strokes.

Division of the Batter. At expedient stages of mixing, the batter was divided into three portions. One-third of the creamed mixture was removed after creaming was completed for the cake to be leavened by air, water vapor, and carbon dioxide. To this, one-third of the flour (previously sifted with the baking powder), one-third of the milk, and one-third of the egg-magma meringue were added. The remaining batter was divided into two portions after the flour, milk, and egg had been added. The air was removed from one portion of the batter by means of a water vacuum pump. Obviously some water was also removed but the fluidity of the batter indicated that enough water remained to furnish leavening by water vapor. The third portion of the batter was used for the cake leavened by air and water vapor.

Baking. The cakes were baked in a thermostatically controlled gas oven maintained at 185°C.

Calculations. Data were taken to study the effect of type of fat and of leavening gases on certain properties of the batters and cakes, and to compute the leavening power of each gas. The specific gravity (3) of the batter was computed from the weight of a measured volume of batter in milliliters. The linespread of Grawemeyer and Pfund (5) was used to determine the consistency of the batters. Cake volumes were determined by seed displacement.

For air and water vapor leavened cakes:

$$\text{Air factor} = \frac{\text{Temperature of batter as it goes in oven} + 273}{\text{Temperature of cake as it comes from oven} + 273}$$

$$\text{Specific volume of batter} = \frac{1}{\text{Specific gravity of batter}}$$

Volume of weighed batter in pan = Specific volume of batter × weight of batter

Total increase in volume of cake = Measured volume of cake − volume of batter

Volume of weighed air-evacuated batter = Specific volume of air-evacuated batter × weight of batter

Volume of air in batter = Volume of batter with air − volume of air-evacuated batter

Volume of heated air = Air factor × volume of air in batter

Volume increase from air expansion = Volume of heated air − volume of air in batter

$$\% \text{ volume increase from air expansion} = \frac{\text{Increase from air expansion} \times 100}{\text{Total increase in volume}}$$

$$\text{Total increase in cake volume} = \frac{\text{Total volume increase at end of baking} \times 100}{\text{Volume of weighed batter}}$$

For air, water vapor, and carbon dioxide leavened batters:

Volume increase from carbon dioxide = Total volume increase of cake − total volume increase of air and water vapor leavened cake

Compute percentages as above.

Palatability. The cakes were rated for grain (30), tenderness (20), texture (smoothness or lack of harshness, 20), and eating quality (equivalent to flavor, if flavor is considered as a combination of aroma, taste, and tactile sensations, 30). The highest possible score for each factor is given in parentheses.

Results and Discussion

Appearance. Typical sections from the centers of the oil cakes are shown in Fig. 1. The air and water vapor leavened cakes, when removed from the oven, had straight sides close against the pan, but immediately shrank until the sides pulled away from the pans and were slightly concave in contour. The crumb of the cake was velvety, tender, moist, and of fine uniform grain, but the top crust did not brown. There was a tendency towards compactness and small soggy spots in some of the cakes.

When baking powder was included in the formula to furnish carbon dioxide in addition to air and water vapor for leavening, the cakes were lighter, less moist, and evenly browned. The cakes had straighter sides than the cakes leavened with air and water vapor. The grain was open and loose, sometimes coarse, and the crumb had a tendency to be harsh and crumbly. Cakes made with oil showed more evidence of harshness and crumbliness than cakes made with the other fats.

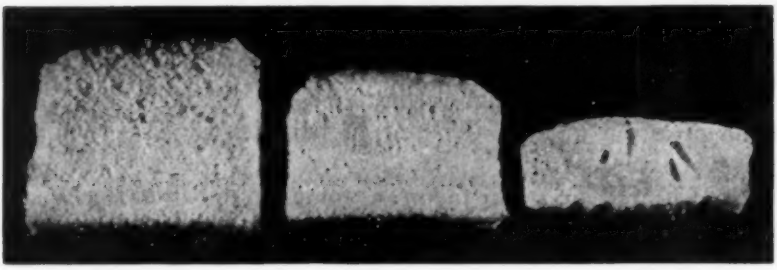

Fig. 1. Cakes containing oil leavened by: (left) carbon dioxide, air, and water vapor; (middle) air and water vapor; (right) water vapor (air-evacuated batter).

The cakes from air-evacuated batters, which were leavened by water vapor, had very small volumes, were distorted in shape, and evidenced little or no cell structure. The interior appeared more like a starch pudding than a cake.

TABLE I

MEAN PALATABILITY SCORES OF CAKES: GRAIN, TENDERNESS, TEXTURE, EATING QUALITY (AROMA, TASTE, TACTILE SENSATION), AND TOTAL SCORES

Fat used	Grain	Tenderness	Texture	Eating quality	Total score
AIR AND WATER VAPOR LEAVENED CAKES					
Butter	23.4	16.0	17.2	24.4	81.0
Oil	24.1	16.0	18.1	24.5	82.7
Hydrogenated lard	21.6	15.9	17.6	22.2	77.4
CARBON DIOXIDE, AIR, AND WATER VAPOR LEAVENED CAKES					
Butter	24.2	17.6	17.1	24.6	83.5
Oil	24.6	17.5	16.1	24.4	82.5
Hydrogenated lard	25.0	16.9	17.2	24.2	82.3
WATER VAPOR LEAVENED (AIR-EVACUATED) CAKES					
Butter	1.2	3.4	0.9	1.3	6.8
Oil	0.0	1.5	0.1	0.5	2.1
Hydrogenated lard	2.0	3.7	1.5	1.4	8.7

Palatability. Statistical analysis of the palatability scores indicated no significant differences among the total scores of cakes with different fats (Table I). There was no significant difference between the palatability scores of cakes leavened by air and water vapor and cakes leavened by carbon dioxide, air, and water vapor. Scores of cakes leavened by water vapor alone were extremely low and the cakes were described as unpalatable. Statistically, the total palatability scores of the water vapor leavened cakes were lower (the differences were highly significant) than total scores of cakes leavened with (1) air and water vapor or (2) carbon dioxide, air, and water vapor.

Average texture scores of cakes leavened by air and water vapor and of cakes leavened by carbon dioxide, air, and water vapor showed only slight differences, but the differences favored the cakes leavened by air and water vapor. Although the amount of baking powder used in this study was the lowest quantity found desirable in an earlier study in this laboratory, the cakes tended to have a coarse, crumbly, and harsh texture typical of too much baking powder. The texture scores of all cakes from air-evacuated batters averaged less than two.

Average grain ratings of cakes leavened by air and water vapor were always slightly lower than the corresponding cakes leavened by carbon dioxide, air, and water vapor, but decidedly higher than ratings of cakes from the air-evacuated batters.

Average eating quality scores were similar for the two groups of cake leavened by air and water and by carbon dioxide, air, and water vapor. In contrast, the flavor scores of the cakes leavened by water vapor were extremely low; these cakes were considered unpalatable (Table I).

Cake Volumes. A striking difference was evident in cake volumes obtained with different leavening agents (Figs. 1 and 2 and Table II). Volumes of cakes leavened by carbon dioxide, air, and water vapor were always largest; those leavened by air and water vapor were intermediate; and those leavened by water vapor alone were the smallest. These differences were highly significant statistically. Comparison of the individual fats showed a highly significant difference between the mean volumes of oil cakes (423.4 ml.) and butter cakes (388.7 ml.), a significant difference between means of oil cakes and hydrogenated lard cakes (402.1 ml.), but no significant difference between hydrogenated lard and butter cakes.

Leavening Attributed to Air. The computations for leavening by air have been based on the assumption that the air incorporated in the batter was 100% effective as a leavening agent. Such was not the case. The maximum expansion of the batter was not the same as the measured volume of the cake (taken when cooled). Maximum ex-

pansion of the batter usually occurred just before baking was completed. Cakes usually shrank some before removal from the oven and continued to shrink after removal. Since the volume increase attributed to air was the maximum expansion of which the air was capable, obviously the proportion of the volume increase attributed to air of the measured cake volume was larger than actually occurred. In addition, no account was taken of the air and water vapor lost during baking. Nevertheless, the results do represent the maximum amount of increase of which the air is capable.

TABLE II

MEANS OF MEASURED CAKE VOLUME, VOLUME INCREASE OVER THE INITIAL BATTER VOLUME, SPECIFIC GRAVITY, AND VISCOSITY AS LINESPREAD

Cakes	Cake volume, ml.	Total volume increase over initial volume, ml.	Specific gravity	Viscosity (linespread)
BATTERS CONTAINING AIR				
Butter	388.1	88.1	0.80	0.9
Oil	417.5	142.3	0.81	4.7
Hydrogenated lard	366.8	66.8	0.80	0.6
BATTERS CONTAINING AIR AND BAKING POWDER (YIELDING SOME CO_2)				
Butter	514.5	216.8	0.79	0.9
Oil	586.2	286.7	0.77	4.0
Hydrogenated lard	565.0	247.5	0.80	0.5
AIR-EVACUATED BATTERS				
Butter	256.8	24.0	1.03	5.0
Oil	256.8	24.0	1.03	7.2
Hydrogenated lard	291.8	61.6	1.02	2.8

The increases in cake volume attributable to thermal expansion of the occluded air were 19.8, 11.4, and 25.0% for cakes containing butter, oil, and hydrogenated lard, respectively, when they were leavened with air and water vapor; the corresponding volume increases for cakes leavened with carbon dioxide, air, and water vapor were 8.0, 5.6, and 6.7%. The volume increase attributable to air expansion measured in milliliters was nearly the same in all groups of cakes in which air was a leavening agent. In the same order as the percentage increase, the increase in milliliters was 17.4, 16.1, and 16.7, and 17.3, 16.0, and 16.6. This indicates that all cakes had very nearly the same amount of air incorporated in the initial batter. The similarity of the

specific gravities of the batters from the three types of fats (Table II) also supports this view.

If the occluded air in the initial batters had been considered as a part of the cake volume increase, thus using the air-evacuated batter as a basis for computing increase in cake volume attributed to air, then the values would have been 54.5, 38.3, and 66.3% for butter, oil, and hydrogenated lard, respectively. These percentages are computed in the same manner as those of Dunn and White (4) and are more nearly in keeping with the approximate 50% increase in pound cake volume attributed to air by these investigators.

Leavening Attributed to Water Vapor. The remainder of the volume increase of cakes leavened by air and water vapor, after deduction of the volume increase brought about by the thermal expansion of the air, was attributed to water vapor. Since the leavening attributed to air was approximately the same in all cakes of the air and water vapor group, the wide variance in total volume was produced by water vapor, Fig. 2. It seems, therefore, that the effectiveness of water vapor in leavening the cakes varied with the type of fat used. The volume increase brought about by water vapor for cakes with butter, oil, and hydrogenated lard was: (1) for the air and water vapor leavened group 70.7, 126.2, and 50.1, (2) for the carbon dioxide, air, and water vapor leavened cakes 70.1, 126.4, and 50.0, and (3) for cakes leavened by water vapor alone 24.0, 24.0, and 61.6 milliliters. Thus the volume increase, with one exception, was always greatest in the cakes containing oil and least in the hydrogenated lard cakes. The one exception was in the group of water vapor leavened cakes where the increases for butter and oil were the same. The volume increases brought about by water vapor are particularly interesting when contrasted with the volume increases attributed to carbon dioxide.

When water vapor was the only leavening agent the volume increase of the butter and oil cakes was very small. Under the vacuum the batter crept up the side of the cell and had a honeycombed structure. Upon removal of the vacuum, the batter collapsed.

Some of the air-evacuated batters of this study showed no increase in volume during baking, but those in which some air remained (usually those of the hydrogenated lard series, but also including the oil cake shown in Fig. 1) had definite volume increases in baking. Throughout the experimental period difficulty was encountered in removing the air from batters containing hydrogenated lard. The hydrogenated lard had a much higher melting point than butter, and this resulted in batters of high viscosity which probably accounted for at least a part of the difficulty in removing the air from them. Whatever the explanation, the hydrogenated lard batters held their air ten-

aciously and on one occasion the final volume of the cake was within 30 ml. of the corresponding cake of the air and water vapor leavened group. Dunn and White (4) reported no increase in the volume of the air-evacuated pound cake batter of their study, but a decided increase (with poor structure) when only a small amount of air was occluded in the batter.

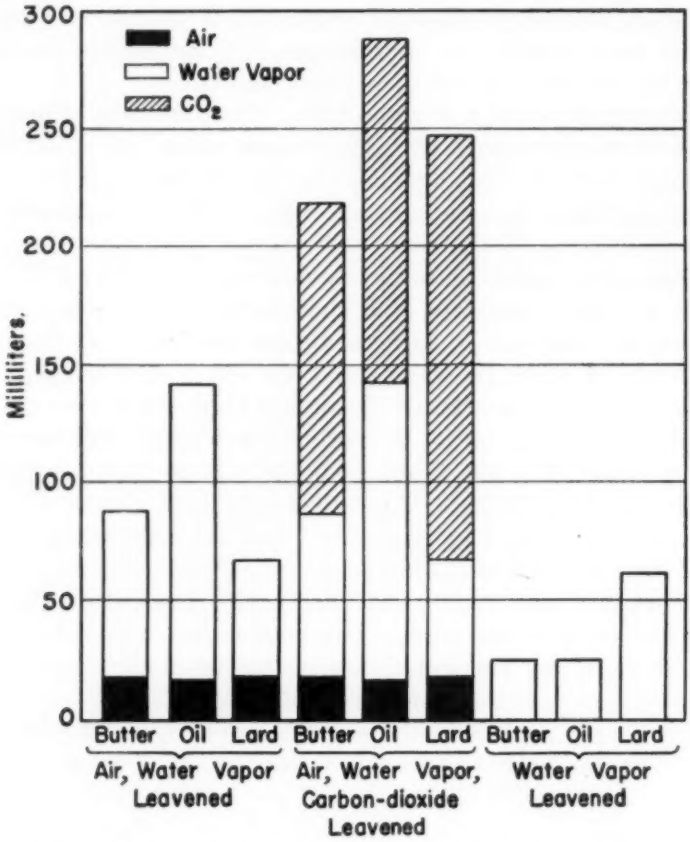

Fig. 2. Cakes made with butter, oil, and hydrogenated lard; leavened by (1) air and water vapor, (2) carbon dioxide, air, and water vapor, and (3) water vapor. The total volume increase over the initial volume of the batter and the proportion of the increase attributed to carbon dioxide, air, and water vapor are shown.

Moisture that was condensed in the pans beneath the water vapor leavened cakes, when the cold cakes were removed from the pans, gave evidence that more vapor had been formed than could escape, either around or through the cake. This could be interpreted as indicating that air spaces are necessary for the vaporization and diffusion of water vapor in the batter.

Leavening Attributed to Carbon Dioxide. After the total cake volume increase above initial volume of the batter was computed, the percentage increase attributed to carbon dioxide was derived by subtracting the total increase of the corresponding air and water vapor leavened cakes. Although this could not be taken as an exact measurement, it seemed a logical relative determination, since the batters and baking conditions were identical except for the addition of baking powder to the carbon dioxide, air, and water vapor leavened cake batters.

The average increases in cake volume attributed to carbon dioxide were 59.7, 50.2, and 73.1% for butter, oil, and hydrogenated lard, respectively. Here again a difference in the effectiveness of a leavening agent is exhibited with the type of fat used. The percentage volume increases with carbon dioxide, in contrast with the volume increase (per cent) attributed to water vapor, were greatest with the firmest fat, the hydrogenated lard, and least with the mobile fat, the oil.

Although the thermal expansion of the air accounted for a relatively small percentage of the volume increase in the cakes, the air incorporated in the batters apparently formed the cell structure into which the water vapor could easily escape. It was interesting that little or no volume increase occurred in the air-evacuated batters, when water vapor was the only leavening agent. This indicates an interdependence among the leavening gases and shows that aeration of cake batters has a function beyond that of increasing cake volume. The presence of air is necessary before the water vapor can function as a leavening gas. These results confirm those of Dunn and White (4) and of Barmore (1). A still greater interdependence among the gases is suggested by Carlin (2). He found that carbon dioxide, in the early stages of baking, does not form new gas cells but expands the existing air bubbles.

Viscosity as Affected by Fats. References are often made in the literature to viscosity of cake batters as an index of cake quality and cake volume. An opportunity was offered in this study to examine the influence on batter viscosity of (1) the mobility of the fat used and (2) the incorporation of gas.

Decided viscosity differences, as measured by linespread, occurred among batters containing different fats. As would be expected from the physical condition of the fats, oil batters showed the greatest fluidity (4.0 to 7.2), butter batters were intermediate (0.9 to 5.0), and hydrogenated lard batters least (0.5 to 2.8). In linespread determinations the batter having the least viscosity covers the greatest surface area of concentric rings one-eighth inch apart. Statistical analysis of the data showed highly significant differences between the viscosity

of oil batters and either of the batters containing the other two fats, and a significant difference between butter and hydrogenated lard.

Viscosity as Affected by Leavening Agents. The batters, on the basis of gas in the batter, comprised three groups, those containing (1) air, (2) air and some carbon dioxide, and (3) the air-evacuated batters. The viscosity differences among these batters were significant, largely because of the removal of air in the third group.

The viscosity means of the batters containing air were of the same order as the viscosity of batters with the different fats. Hydrogenated lard was greatest with 0.6, butter intermediate with 0.9, and oil fell considerably lower with a mean of 4.7. As might be expected, the viscosity means of batters containing carbon dioxide and air (since only a small portion of the baking powder would react to form carbon dioxide at the batter temperature) varied very little from the means of the batters containing only air (Table II).

The viscosity of the air-evacuated batters with all fats was much lower than that of batters containing either air or air and carbon dioxide and made with corresponding fats. This indicates the influence of incorporated gas on batter viscosity, whereas the difference still existing among the batters containing different fats represents the effect of the fat itself on batter viscosity. The order of viscosity means of the air-evacuated batters was hydrogenated lard 2.8, butter 5.0, and oil 7.2.

Thus, under the conditions of this study both the incorporation of gas and the viscosity of the fat had significant effects on the viscosity of the batter. In addition, viscosity was not always a good criterion of cake quality.

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EFFECT OF SYNTHETIC PLANT GROWTH STIMULANTS ON SOME PROPERTIES OF CEREAL PROTEINS AND ON MIXOGRAM PATTERNS [1]

R. H. HARRIS, L. D. SIBBITT, and ORVILLE J. BANASIK [2]

ABSTRACT

The concentration of alpha-sodium naphthyl acetate and sodium phenyl acetate was directly related to the per cent of total protein extracted from rye, wheat, barley, and millet flour, the percentage extracted decreasing in the order in which the cereals are named. The highest concentration of growth stimulant employed was 15.0%, at which point the effect of concentration on per cent of protein extracted appeared to be approaching a constant. The effect of different stimulants on protein solubility varied; sodium naphthyl acetate had the greatest effect. This substance in relatively low concentration apparently tended to coagulate wheat flour protein dispersions in water, while at higher concentrations it increased the solubility of this protein. Sodium naphthyl acetate and 2-4 dichloro-phenoxy sodium acetate were very effective precipitating agents for 0.1 *N* acetic acid dispersions of gluten, removing approximately 98.0% of dispersed protein at a concentration of 1400 mg. per 100 ml. of dispersion and greatly decreasing the relative viscosity. From the limited data collected, the final effect on solubility appeared to be independent of the individual stimulant employed.

The initial influence of stimulants on mixogram pattern of hard red spring wheat flours was to increase the strength of the curve; but beyond a concentration of approximately 2.0% of weight of flour used, a deleterious effect which increased with addition of stimulant was noticeable. Different wheat varieties were affected in the same manner.

It is apparent that synthetic plant growth stimulants have a marked effect on some properties of cereal proteins, and that this effect varies with the stimulant.

A large number of organic chemicals is known to promote the growth of plants. These substances contain either an unsaturated or an aromatic ring, and a carboxyl (or a group readily converted by the plant to a carboxyl) separated from the ring by at least one carbon or oxygen atom. Three naturally occurring substances have been isolated which promote growth. These are auxentriolic acid (auxin a), auxenolonic acid (auxin b), and indoleacetic acid (9). Not only do these materials promote growth, but under certain conditions they may inhibit growth, leading to their use in destroying weeds.

Grace (2) found that 5.0% neutral sodium salt solutions of the group of chemicals comprising synthetic plant growth stimulants dispersed undenatured wheat gluten. Sodium naphthyl acetate ap-

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peared to be the most efficient, dispersing essentially as large a proportion of the gluten as sodium hydroxide and 10.0% sodium salicylate. Grace noted that this property might be related to the mechanism of plant responses to these compounds. No attempt was made to determine the effect of growth stimulant concentration on quantity of gluten extracted.

The present work consists of a study of the effect of plant growth stimulants on the quantity and properties of protein dispersed from several cereals, as well as the influence of stimulant on the mixogram pattern of hard red spring wheat flour.

Materials and Methods

The flours employed in this study were approximately 95.0% long patents which had been experimentally milled from hard red spring wheat varieties grown on experimental plots in 1946. The varieties were Thatcher, Pilot, Mida, and Cadet, each grown at branch experiment stations located at Dickinson, Langdon, and Edgeley. These included a range of protein from 13.2 to 15.4% and comprised three mixogram types, strong, medium, and weak. A composite sample was prepared from a blend of several commercially important hard red spring wheat varieties grown at several points in North Dakota. The barley variety was Manchuria (12.5% protein) while the rye was Washington Imperial (7.2% protein), both grown in the Experiment Station plots at Fargo in 1945. The millet was a yellow variety, Proso (8.6% protein), purchased from a grocery store. These three protein values ($N \times 6.25$) are expressed on a 13.5% moisture basis. All samples were sound and free from damage.

The wheats were milled by the method described by Sibbitt, Scott, and Harris (8) for the Allis mill, while the other three cereals were milled by suitable modifications of this procedure. The millroom was maintained at approximately 70°F. and 65% relative humidity.

The effect of growth stimulants on protein solubility was determined employing 5 g. of flour in 100 ml. of distilled water containing the required concentration of stimulant and agitating gently for 6 hours in a rotary shaker (4). The flour suspensions were centrifuged and the protein content of the decanted solution determined by the Kjeldahl-Gunning method. When examining the coagulating action of growth stimulants, suitable additions of the active substance were made to the flour suspensions at the beginning of shaking. Six hours, with gentle shaking, was allowed for the stimulants to act on the flour protein. The dispersions were then centrifuged, and the nitrogen content and viscosity of the supernatant liquid determined. Viscosity determinations were made with the Ostwald pipette at 25°C. $\pm$ 0.05°C.

No effort was made to standardize the pipette since it has been shown that relative rather than absolute viscosity values are of major importance in biological chemistry (1, 7). The pH of the dispersions was obtained by a Beckmann pH meter.

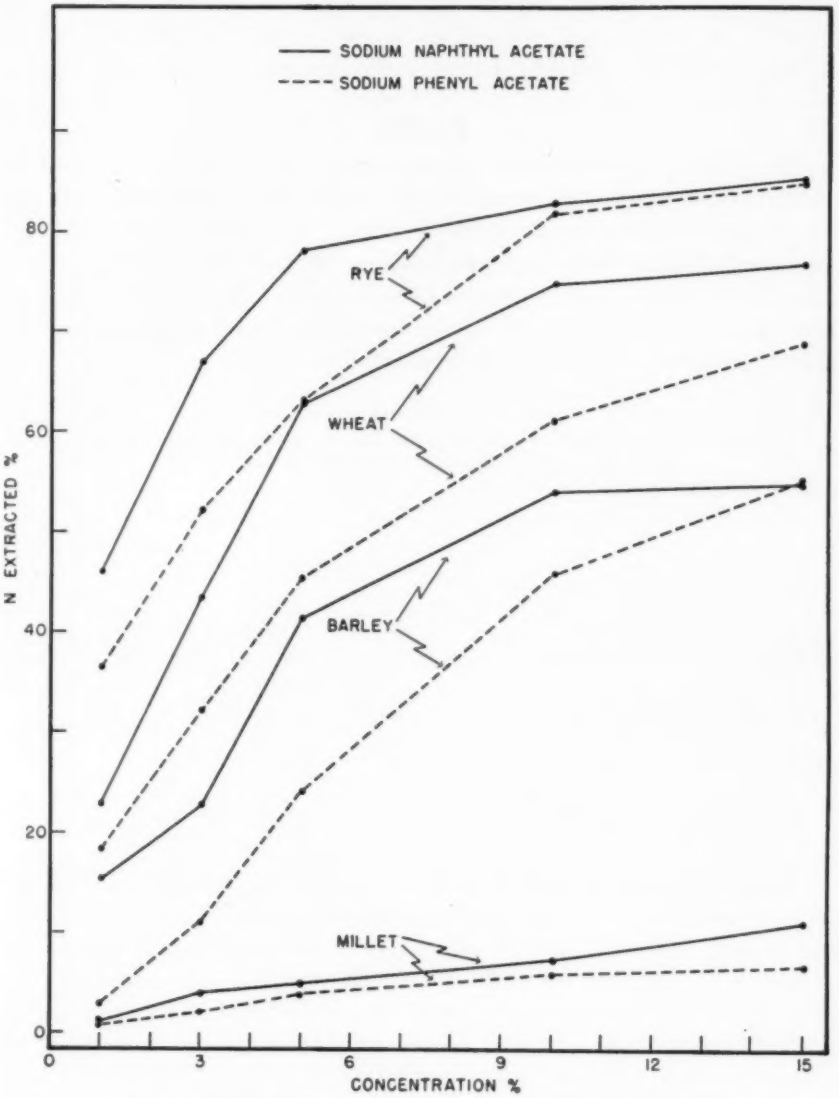


Fig. 1. Solubility of four cereal flour proteins in various concentrations of sodium naphthyl and sodium phenyl acetates.

The gluten dispersions were made by the method described by Harris and Johnson (5) using the Waring Blendor and 0.1 *N* acetic acid. After removal of undispersed material by centrifuging, suitable

aliquots were taken for protein precipitations. The dry salt was added to the dispersion.

The mixograms and their dimensions were obtained by the procedure described by Harris (3) using flour and distilled water at the absorption found when baking the flours. The stimulant in the dry form of its sodium salt was added to the water immediately before mixing. Suitable concentrations were selected from preliminary determinations.

Results

The results secured from the extraction and viscosity determinations are presented, with one exception, in the form of curves, with the actual values shown as dots in the figures. This mode of presentation facilitates comparisons, and emphasizes the fact that precise values cannot be expected in this type of investigation. A table of mixogram dimensions is included to enable comparisons to be made among the different treatments with growth stimulants.

TABLE I

COMPARATIVE PROPORTIONS OF THE TOTAL NITROGEN EXTRACTED FROM CEREAL FLOURS BY DIFFERENT REAGENTS

Extractant[1]	Nitrogen extracted			
	Rye	Wheat	Barley	Millet
	%	%	%	%
Sodium salicylate, 10.0%	77.0	78.7	56.4	12.7
2-4 Dichloro-phenoxy sodium acetate, 1.1%	50.7	49.7	19.7	8.0
2 Naphthoxy sodium acetate, 1.3%	40.0	35.0	16.7	7.3
2-4-5 Trichloro-phenoxy sodium acetate, 0.6%	38.0	16.0	15.4	2.6
Distilled water	37.0	15.8	15.6	3.0

[1] Growth stimulant solutions were all saturated.
Note: pH of extracts was 6.8.

Effect of Plant Growth Stimulants on Protein Solubility. The results secured from treating the four cereal flours with various concentrations of sodium naphthyl acetate and sodium phenyl acetate are shown in Fig. 1. As noted by Grace (2) the former compound consistently extracts more protein than the phenyl salt. The concentration-extraction curves begin to level off after 5.0% concentration for sodium naphthyl acetate, and after 10.0% for sodium phenyl acetate. Rye protein was most easily extracted by both substances, with wheat second, and barley third. The proportion of protein removed from millet was very small. At the higher solvent concentrations there appeared to be some tendency for the curves to converge. Grace used the 5.0% level, where the curves are more divergent, and differences

between the two stimulants are most marked. The pH of all dispersions was approximately 6.8.

In Table I are data showing the per cent of nitrogen extracted from the flours by various substances, including three growth stimulants.

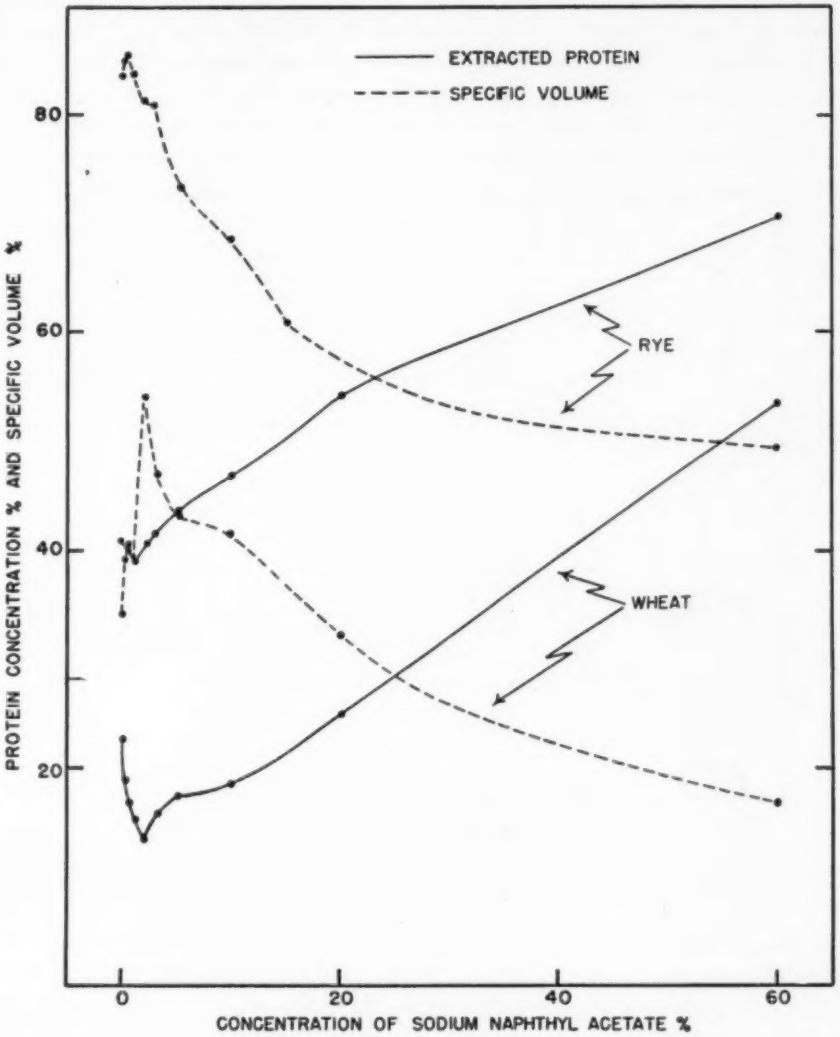


Fig. 2. Effect of concentration of sodium naphthyl acetate on protein extraction and specific volume of the protein micelle. Concentration of stimulant expressed as per cent of flour.

The concentrations are different in each solution because of the varying solubilities of the hormones, so direct comparisons cannot be made in regard to the relative solvent power. The relative insolubility of the proteins of millet is very marked, only 3.0% of the total nitrogen being

extracted by distilled water, while rye has 37.0% removed by this solvent.

Fig. 2 shows the effect of various concentrations of sodium naphthyl acetate solution on the amount of protein extracted from wheat and rye flours, as well as the influence on the specific volume of the protein particle in dispersion (6). This latter effect was investigated because it was found that the growth stimulants induced remarkable changes in the mixogram pattern of bread wheat flours (Fig. 6). At low initial concentrations there is a decrease in the amount of protein extracted for both wheat and rye, followed by a regular increase after a con-

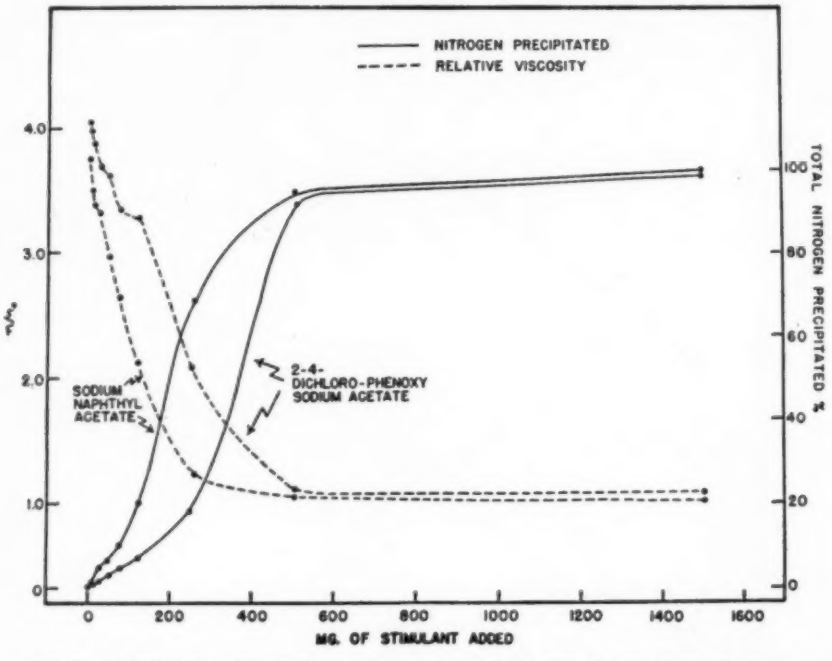


Fig. 3. Relations between stimulant concentration and per cent of total dispersed protein precipitated from gluten dispersions in 0.1 *N* acetic acid. Effect of stimulant on the relative viscosity, η/η_0, is also shown.

centration of 0.25% was attained. Specific volume is affected in the opposite direction: first there is an increase in particle size as coagulation proceeds, and solubility decreases, while later the particle size progressively decreases as solubility rises. The initial effect on particle size is quite marked for wheat. From these results it is concluded that the initial effect of stimulants is to depress solubility by a coagulative influence, followed by a dispersive action at higher dosages.

Effect of Plant Growth Stimulants as Protein Precipitating Agents. Fig. 3 shows the results secured from additions of sodium naphthyl acetate and sodium dichloro-phenoxy acetate to dispersions of gluten

protein in 0.1 *N* acetic acid. The stimulants cause increasing precipitation of protein from the dispersion until a dosage of 500 mg. per 100 ml. is reached, after which further increase in reagent concentration has little influence on the quantity of protein removed. Sodium naphthyl

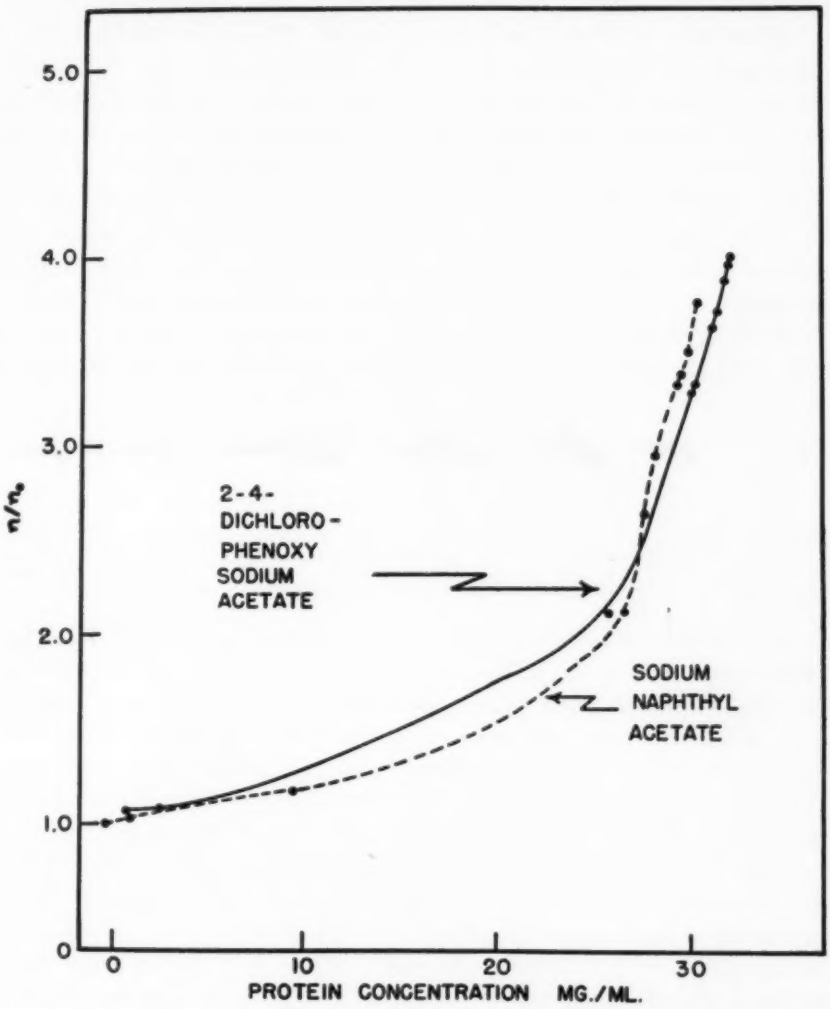


Fig. 4. Relation between relative viscosity and protein concentration in gluten dispersions in 0.1 *N* acetic acid. Data secured by precipitating the protein by graduated additions of two growth stimulants (Fig. 3).

acetate has a slightly greater effect than sodium dichloro-phenoxy acetate until the maximum is reached, when the two salts become practically identical in their effects. The effect of the growth stimulants on relative viscosity is approximately the same as on concentration, viscosity decreasing as stimulant concentration increases until a

limit is reached beyond which there is little further increase as more stimulant is added.

The relation between viscosity and protein concentration shown in Fig. 4 reveals a marked fall in viscosity as the quantity of protein in dispersion decreases. The rather sharp inflection in the viscosity curve at approximately 25 mg./ml. concentration of protein might indicate a change in the type of protein fraction precipitated. The "glutenin" fraction, which is thought to include the larger particles, would be present in the dispersion before much protein had been removed by the growth stimulant, and would tend to affect viscosity more than other protein fractions which consist of smaller particles. The curves for the two stimulants, sodium naphthyl acetate and 2-4 dichloro-phenoxy sodium acetate, are practically superimposed, and show the familiar curvilinear relationship between protein concentration and viscosity.

Effect of Plant Growth Stimulants on Mixogram Patterns. Fig. 5 shows the effect of several plant growth stimulants on the mixogram

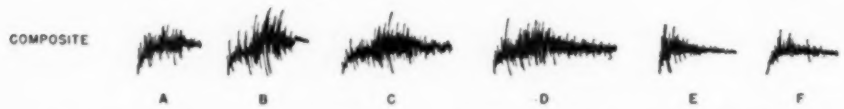
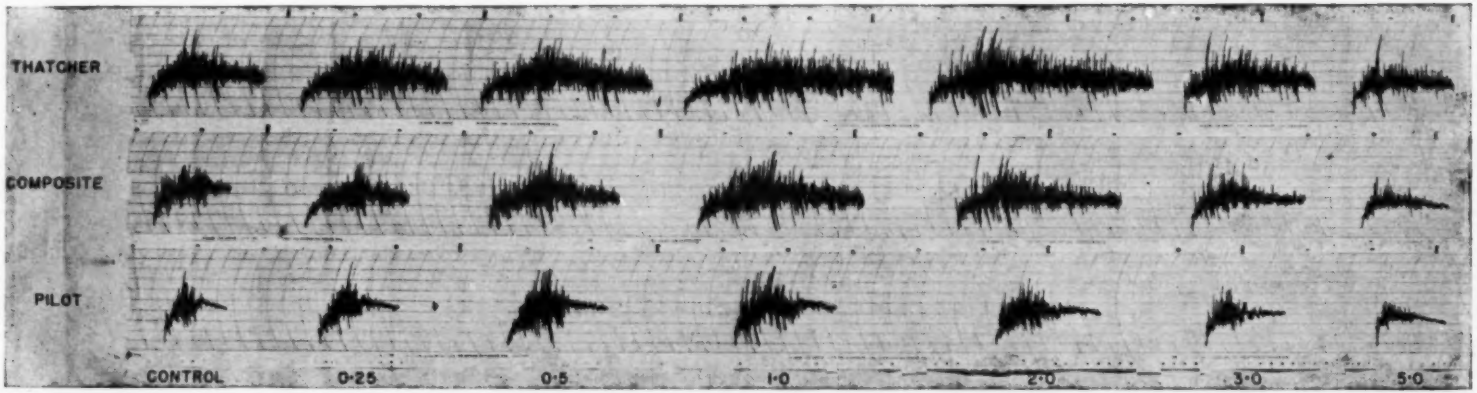


Fig. 5. Effect of different synthetic plant growth stimulants on the mixogram pattern of a hard red spring wheat flour. Concentration expressed as per cent of flour. A, control; B, 2 naphthoxy sodium acetate, 0.78; C, 2-4-5 trichloro-phenoxy sodium acetate, 0.36; D, 2-4 dichloro-phenoxy sodium acetate, 0.66; E, sodium phenyl acetate, 5.0; F, sodium naphthyl acetate, 5.0.

pattern of a flour experimentally milled from a number of hard red spring wheat samples. 2 Naphthoxy sodium acetate, 2-4-5 trichloro-phenoxy sodium acetate, and 2-4 dichloro-phenoxy sodium acetate appeared to increase the strength of the curve in the order named. Sodium phenyl acetate and sodium naphthyl acetate, however, when present in 5.0% concentration, markedly reduced the strength of the mixogram. The concentration of the first three substances employed was low (Fig. 5), owing to their poor solubility in water. This difference in concentration will significantly affect the curve characteristics, and operates against making direct comparisons regarding the effects of the five growth stimulants. However, it was later found that treatments of sodium naphthyl acetate in the 0.5% to 1.0% concentration range (Fig. 6) gave somewhat similar curve patterns with the same flour. There is little doubt that increasing the concentration of the first three salts would also cause a reduction in the strength of the mixogram. These data indicate that plant growth stimulants strongly affect mixing requirements. The strength of the effect tends to be related to the physiological activity of the compound in conformity with *a priori* expectations.



Flg. 6. Relations between concentration, as per cent of flour, of sodium naphthyl acetate, and the mixogram patterns of three hard red spring wheat flours.

Fig. 6 represents the influence of different concentrations of the most active stimulant, sodium naphthyl acetate, on the mixogram pattern of three hard red spring wheat flours possessing quite different mixing properties. The wheat protein content of these samples was as follows: Thatcher, 14.9%; composite, 12.9%; and Pilot, 13.6%. The normal mixograms secured from these flours are shown at the left of the figure; the differences in pattern type are quite evident. The initial effect of the stimulant was to increase all four of the dimensions measured for the three flours. This increased strength persisted until a concentration of 2.0% was reached for Thatcher, and 1.0% for the composite flour and for Pilot, although there was very little difference between the 0.5% and 1.0% treatments for the latter. Beyond this point all curves progressively weakened as stimulant concentration increased. In view of the effect of these substances on protein solubility in aqueous solution it seems logical to assume that the initial increase in the strength of the curves is due to a coagulative effect on the protein, followed beyond the optimum point by a dispersive action which would tend to weaken the physical properties of protein and result in a decrease in the strength of the curve.

The average dimensions of four mixogram properties secured from flours representing four hard red spring wheat varieties grown at three North Dakota stations in 1946 are shown in Table II. These varieties cover the range to be found in mixogram properties among spring wheats; Cadet has probably the strongest pattern, with Thatcher second in strength, and the remaining two varieties weaker in type. One per cent of stimulant increases the value for all four properties for all the four wheats, the greatest effect being probably in dough development. The next treatment, 2.0%, slightly decreased dough development from the 1.0% values. For dough stability, there was a decided increase, while the other two dimensions were not greatly altered from the previous values. The highest concentration of stimulant, 5.0%, greatly reduced all mixogram properties, and yielded lower values than the control, though in some instances the latter differences were not large. All flours appeared to be affected in much the same manner, regardless of curve type.

The data show that the character of the mixogram may be markedly changed by the addition of plant growth stimulants. A flour with weak mixing properties may approach a strong type flour in behavior upon suitable treatment with these substances. Conversely, a flour with too strong mixing requirements could exhibit weaker characteristics by a heavier treatment. It is believed that the baking quality of flour could be greatly changed by this class of substances provided the

TABLE II

EFFECT OF SODIUM NAPHTHYL ACETATE ON THE MIXOGRAM DIMENSIONS OF FOUR HARD RED SPRING WHEAT FLOURS

Wheat variety	Conc. of stimulant[1]	Dough development	Dough stability	Curve height	Curve width
	%	*cm.*	*cm.*	*cm.*	*cm.*
Thatcher	0	4.6	4.9	7.6	1.1
Thatcher	1.0	8.7	8.0	7.7	1.6
Thatcher	2.0	7.5	9.5	7.8	1.8
Thatcher	5.0	3.9	3.9	6.8	0.9
Pilot	0	4.1	3.8	7.1	0.9
Pilot	1.0	6.0	4.6	7.8	1.1
Pilot	2.0	6.2	6.3	7.4	1.7
Pilot	5.0	2.9	3.1	6.3	0.6
Mida	0	3.5	3.5	7.1	0.9
Mida	1.0	5.9	4.5	7.7	1.3
Mida	2.0	5.2	6.5	7.5	1.6
Mida	5.0	2.9	3.6	6.1	0.8
Cadet	0	5.7	6.3	6.8	1.1
Cadet	1.0	10.7	9.8	7.8	1.5
Cadet	2.0	10.2	12.0	7.6	1.7
Cadet	5.0	4.4	6.0	6.6	1.0
MEANS FOR ALL VARIETIES					
	0	4.5	4.6	7.2	1.0
	1.0	7.8	6.7	7.8	1.4
	2.0	7.3	8.6	7.6	1.7
	5.0	3.5	4.2	6.5	0.8

[1] Expressed as per cent of quantity of flour used (25 g. on 13.5% moisture basis).

lethal effect of the stimulant on the yeast and other enzyme systems could be eliminated.

The data show that synthetic plant growth stimulants exert a very marked effect on the proteins of wheat, rye, and barley. Some stimulants in aqueous solution are very efficient extractives for wheat gluten, but function as precipitating reagents for gluten dispersed in 0.1 *N* acetic acid. At relatively low concentrations, they act as coagulative agents for water-soluble protein, and at higher concentrations they perform as dispersive substances. Their effect as growth stimulants at concentrations very much below these employed here suggest that their principal action on plant growth is through enzyme systems, rather than on the plant proteins *per se*.

Acknowledgment

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A REVISED METHOD FOR THE DETERMINATION OF BROMATE IN BROMATED FLOURS [1]

L. R. JOHNSON [2] and A. W. ALCOCK [3]

ABSTRACT

The method of Hoffer and Alcock (1) for the determination of bromate in wheat flour has been modified with the object of improving the replicability and accuracy of the results. As in the original method, the extracted bromate is allowed to react with potassium iodide in the presence of starch and the intensity of the color of the resulting starch-iodine solution is measured in a spectrophotometer. With flours containing 5 p.p.m. of bromate the modified method gives average recoveries of 101.5%; with flours containing 10–20 p.p.m., 97%; and with flours containing 35 p.p.m., 95%. The mean difference between duplicate results obtained on different days was 1.14 p.p.m. This difference was influenced very little by the bromate content of the flour, but is believed to depend very largely upon the granulation of the bromate and its distribution.

The procedure described by Hoffer and Alcock (1) for the determination of bromate in flour has not proved generally satisfactory. A critical study of the method made in this laboratory showed that the results were influenced by small differences in technique such as may easily occur between one analyst and another. After trying numerous changes in an effort to secure more consistent results, the following modified procedure was finally adopted.

[1] Manuscript received March 10, 1948.
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Materials and Methods

Apparatus

(1) 50 ml. centrifuge tubes, without lip.
(2) Coleman Universal Spectrophotometer, Model 11, and optically matched round cuvettes of 1.7 cm. diameter.

Reagents

(1) 25% potassium chloride solution.
(2) Iodine, stock solution. Dissolve 0.35 g. of iodine in 7 ml. of ethyl alcohol and dilute to 1 liter with 1% potassium iodide solution. This solution should be protected from the light.
(3) Potassium chloride solution, 25% in 2% acetic acid.
(4) Celite filter aid, analytical grade.
(5) Standard potassium bromate solution, 5.0 μg. per ml. Prepare as required from a solution containing 100 μg. per ml. which is stable.
(6) 0.5% starch solution, prepared by the following method as outlined by Platner (2): While stirring add approximately 30 ml. of a 20% sodium hydroxide solution to a suspension of 2 g. of soluble starch in 300 ml. of water and allow to stand for 1 hour. Neutralize with concentrated hydrochloric acid using litmus as an indicator and then add 1 ml. of glacial acetic acid. Dilute to 400 ml. with water. This solution can be used for an indefinite period.
(7) 1% potassium iodide solution.
(8) Iodine solution, approximately 17 μg. per ml. Dilute approximately 2.5 ml. of stock iodine solution to 50 ml. Prepare fresh daily.
(9) 5% sulfuric acid.

Procedure. Weigh 7 g. of flour into a 50 ml. centrifuge tube and add 40 ml. of a mixture consisting of approximately 37.4 volumes of 25% potassium chloride solution and 2.6 volumes of the stock iodine solution. This mixture should be made up just before use. Stopper the centrifuge tube with a rubber stopper and thoroughly disperse the flour by shaking vigorously for 10–15 seconds. Allow to stand for a few minutes and shake again for about 10 seconds. The interval may be utilized for the extraction of other samples in the set. Time of standing is not critical but the shaking must be sufficient to disperse the flour to allow for the solution of the bromate. Centrifuge at 2400 r.p.m. for 10 minutes. Dilute 5 or 10 ml. of the supernatant liquid (or less if the flour contains in excess of 50 p.p.m. of bromate) to 35 ml. with 25% potassium chloride solution in 2% acetic acid and, after

mixing, add from a spatula about 0.8 g. of Celite (as judged by the eye). Stir vigorously with a stirring rod and centrifuge at 2400 r.p.m. for 5 minutes. Pour off all the clear extract and then prepare the following mixtures:

Tube 1 (Reference Solution)	Tube 2	Tube 3
3 ml. distilled water	1 ml. distilled water	1 ml. standard bromate soln.
1 ml. starch soln.	1 ml. starch soln.	1 ml. starch soln.
10 ml. flour extract	10 ml. flour extract	10 ml. flour extract
3 ml. potassium iodide soln.	3 ml. potassium iodide soln.	3 ml. potassium iodide soln.
1 ml. dilute iodine soln.	1 ml. dilute iodine soln.	1 ml. dilute iodine soln.
	2 ml. 5% sulfuric acid	2 ml. 5% sulfuric acid

Each addition is made in rotation, as rapidly as convenient and in the order given. Allow an interval of 2 minutes between the acid additions to tubes 2 and 3, and exactly 4 minutes after adding the acid read the optical density at a wave length of 575 mμ. The difference in optical density between tubes 2 and 3 is due to 5 μg. of bromate and, when 10 ml. of the first extract is taken, the concentration of bromate in the flour is obtained as follows:

Concentration of bromate,

$$\text{p.p.m.} = \frac{A}{B} \times \frac{35}{10} \times \frac{40}{10} \times \frac{1}{7} \times 5 = \frac{10A}{B}$$

A is the optical density of the unknown in tube (2),
B is the difference in optical density between tubes 3 and 2.

While spectrophotometer readings are being made on one extract, the decanted clear extracts from other samples in the same set can be allowed to stand. They should not, however, be left too long, since slow losses of bromate occur as shown by the following results:

Hours standing	Bromate recovery
0	5.3 p.p.m.
2	5.1 p.p.m.
4	5.0 p.p.m.
6	4.9 p.p.m.

Results and Discussion

The first objective of the efforts to improve the original method was to secure clear extracts as free as possible from flour starch. This was accomplished by extracting with neutral potassium chloride solution, diluting an aliquot with potassium chloride in acetic acid, and removing the precipitate then formed by centrifuging after the addition of Celite. Dilution of the original extract with acid potassium chloride solution is necessary, otherwise precipitation would occur on acidifying at the color development stage. The characteristics of the color produced on adding starch and iodine to clear extracts, obtained in this manner from a variety of flours of different grades and different ages,

were very uniform. With all extracts the maximum light absorption peaks overlapped in the neighborhood of 575 mμ. and any lack of definition of the peaks due to cloudiness was avoided.

Hoffer and Alcock noted that when their method was applied to unbromated flours, optical density readings equivalent to 0.6 p.p.m. of bromate were obtained. The present studies, however, showed the magnitude of this "blank" to be greatly influenced by the time and vigor of shaking. This particular difficulty, which had undoubtedly been responsible for some of the discrepancies in the results of other workers, disappeared on changing the method of extraction. A "blank" was still obtained with unbromated flours but it was small; and in spite of wide variations in the amount of shaking, its value, expressed as μg. of bromate in the mixture used for color development, only varied from 0.1 to 0.25.

The new extraction method has the further advantage of permitting the use of a larger ratio of flour to extractant, thus reducing the sampling error. Because of the low dosages of bromate used, and the differences in specific gravity and particle characteristics between flour and bromate, it is believed that this error can be quite large.

With the adoption of neutral potassium chloride solution as the bromate extractant, it became necessary to discontinue the use of potassium permanganate since it was no longer reduced. Low bromate recoveries were now obtained. It was then found that on adding increments of a dilute iodine solution to the extract of an unbromated flour, the first addition produced a smaller increase in optical density than subsequent increments. The same thing occurs when iodine is released by increments of bromate; the first increment of bromate gives a low reading. It was, therefore, decided to add a small quantity of iodine to each of the tubes immediately before the addition of acid, and thus ensure that all the bromate from the flour was effective in increasing the color intensity. The exact quantity of iodine added is not important, but it is convenient to use an amount equal to that released by 2 to 2.5 μg. of bromate.

Upon the introduction of this step bromate recoveries became slightly high. By using extracts of unbromated flours, the high recoveries were found to be due to the fact that the intensity of the starch-iodine color in the tube containing added acid was greater than that in tube 1 containing the reference solution to which no acid is added. As this was not the case when potassium chloride in 2% acetic acid was substituted for flour extract, it was assumed, as a working hypothesis, that soluble substances from the flour took up small quantities of iodine in tube 1, and that this did not occur in the tube containing added acid. In the hope of saturating these soluble substances

with iodine, an excess of iodine was added to the extractant. Whether the hypothesis is correct or not, the use of iodine in the extractant resulted in more satisfactory bromate recoveries. The amount of iodine added is not critical. All that is necessary is to have a visible excess that is adsorbed on the flour and is subsequently removed on centrifuging.

As iodine is slowly liberated when the mixed reagent, used in the original method, is allowed to stand, it was decided to add the acid

TABLE I

RESULTS OF BROMATE DETERMINATIONS ON MIXTURES OF FLOUR AND BROMATE

Grade of flour	Bromate added, p.p.m.	Bromate found,[1] p.p.m.		Mean, p.p.m.	Mean recovery, %
Patent	5	4.6	4.7	4.65	93.0
Patent	5	4.5	5.6	5.05	101.0
Straight	5	5.0	5.5	5.25	105.0
Clear	5	4.7	5.8	5.25	105.0
Means				5.05	101.0
Patent	12	10.9	11.8	11.35	94.6
Patent	12	10.9	11.5	11.2	93.3
Straight	12	10.6	13.4	12.0	100.0
Clear	12	12.4	12.4	12.4	103.3
Means				11.75	97.9
Patent	20	19.0	20.1	19.55	97.8
Straight	20	19.0	20.2	19.6	98.0
Clear	20	17.7	20.1	18.9	94.5
Means				19.35	96.8
Patent	35	34.8	35.0	34.9	99.7
Straight	35	34.1	35.9	35.0	100.0
Clear	35	29.2	31.2	30.2	86.3
Means				33.4	95.3

[1] Duplicate determinations on different days.

and iodide separately. At the same time the concentration of acid was increased in order to speed up the reaction with bromate. After an interval of 3 minutes from the time the stronger acid is added, further increases in optical density take place very slowly. But because they do take place and because there is a very slow fading of the color of the reference solution in tube 1, this short color development time is desirable. Sulfuric acid was substituted for hydrochloric acid because it gave a lower reagent blank. The reagent blank, which should be checked from time to time, should not exceed a value equiva-

lent to 0.25 μg. of bromate in the mixture of solutions used for color development. If it does, fresh sulfuric acid should be prepared.

The revised method suffers from the same limitation as the original in that it cannot be used when iodates or other substances which release iodine from iodide are present in the flour.

To check the revised method a master mix was prepared by mixing 999 g. of patent flour and 1 g. of potassium bromate in a McLellan mixer for 4 hours. From this, samples of patents, straight and clear,

TABLE II

RECOVERIES OF BROMATE ADDED TO THE EXTRACTANT

Grade of flour	Bromate added, p.p.m.	Bromate found,[1] p.p.m.		Mean, p.p.m.	Mean recovery, %
Patent	5.7	5.7	5.8	5.75	100.9
Patent	5.7	5.5	5.6	5.55	97.4
Straight	5.7	5.8	5.8	5.8	101.2
Clear	5.7	6.1	6.3	6.2	108.8
Means				5.8	102.2
Patent	11.4	10.9	11.1	11.0	96.5
Patent	11.4	10.6	10.9	10.75	94.3
Straight	11.4	11.0	11.3	11.15	97.8
Clear	11.4	11.0	11.3	11.15	97.8
Means				11.0	96.6
Patent	34.2	32.4	—	32.4	94.7
Patent	34.2	32.1	32.7	32.4	94.7
Straight	34.2	32.2	32.6	32.4	94.7
Clear	34.2	32.2	32.5	32.35	94.6
Means				32.4	94.7

[1] Duplicate determinations on different days.

were made up to contain 5, 12, 20, and 35 p.p.m. of bromate, the mixing of these samples being carried on for 30 minutes. The results are given in Table I.

In a second series, the bromate was added in solution with the potassium chloride-iodine mixture. The results are reported in Table II. The mean difference between the duplicate results was only 0.3 p.p.m. as compared with 1.14 p.p.m. between those given in Table I. Errors involved in diluting the neutral potassium chloride extract, clarifying the diluted solution, and developing the color are very small. Determinations of the bromate present in 10 ml. aliquots of neutral potassium chloride extracts showed that the sum of these errors is responsible for a difference of not more than 0.1 p.p.m. in the results of duplicate bromate determinations on flour. With smaller aliquots

the difference due to these errors would be correspondingly increased because of the larger factor used in the calculation.

From the fact that average recoveries were just as good when bromated flours were analyzed as when the bromate was added with the extractant, it would appear that errors in extraction are not responsible in any substantial measure for the poorer replicability when dealing with bromated flours. The most probable explanation seems to lie in the sampling. After the work reported above had been completed, it was found that the bromate used commercially as a flour improver varies widely in granulation. The material used in this study was of intermediate fineness but the granulation was not uniform. It contained a small fraction of relatively large crystals which failed to pass a 16XX sieve. Some idea of the effect of granulation and the difficulty of dispersing bromate uniformly can be obtained by dropping acidified potassium iodide on smooth flour surfaces and noting the distribution and size of the black spots. A few determinations on flours containing bromate in the form of a very fine powder gave duplicate results which agreed as well as those reported in Table II. This study suggests that particle size will have to be considered in any future work on the bromate determination, for it not only influences the sampling error but is likely to affect recoveries in methods involving sedimentation.

The greater variability in the recovery figures for dry mixed samples is attributed largely to lack of homogeneity of the master mix. These variations are smoothed out in the averages, and average recoveries at the 5, 12, and 35 p.p.m. levels were practically the same in both series. With increasing bromate content, recoveries were lower, averaging 95% at 35 p.p.m.

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EFFECT OF HEREDITY ON THE NIACIN AND PANTOTHENIC ACID CONTENT OF CORN [1]

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ABSTRACT

Analyses for niacin and pantothenic acid were made on the inbred lines and single-cross hybrid components of four double-cross hybrids reported in an earlier study. The niacin content of both single- and double-cross hybrids appeared related to that of the inbred lines. It was more difficult to discern a hereditary influence on pantothenic acid, probably because of environmental differences.

Analyses of three inbred lines crossed in various combinations for the study of heterozygosity showed that the two vitamins may behave differently as to inheritance.

Analyses of 36 samples from a group of early, uniform single-cross hybrids, involving nine inbred lines and all grown in approximately the same environmental conditions, showed definite hereditary influence on both niacin and pantothenic acid.

The results in general confirm previous conclusions that the elaboration of both vitamins is influenced by hereditary factors but that the influence on niacin is less subject to modification by environmental factors than is the influence on pantothenic acid. It is suggested that the niacin content of corn can be increased by breeding.

It is recognized that the chemical composition of plants may be affected to a marked extent by both hereditary and environmental factors. Very little is known, however, as to the part which such factors play in the elaboration of vitamins.

Rather wide variations in the amount of several members of the vitamin B complex in corn have been reported by Burkholder *et al.* (2), Teply *et al.* (6), and Ellis and Madsen (4). Recently Aurand and coworkers (1) reported that heredity influenced the carotene content of single-cross corn hybrids. Doty and associates (3) found some indication that the amounts of the various amino acids present in several single-cross hybrids were related to the genetic constitution of the plant.

Previous studies in our laboratory (Hunt *et al.*, 5) showed that both niacin and pantothenic acid in double-cross corn hybrids were influenced significantly by hereditary and environmental factors. The elaboration of pantothenic acid was affected much more by environmental factors than was the elaboration of niacin.

The results in general indicated the need for further investigation of the inbred lines and single-cross hybrids which entered into the

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genetic make-up of the double-cross hybrids. The present report deals with the niacin and pantothenic acid content of various inbred lines and related single- and double-cross hybrids.

Materials and Methods

Analyses were made of the inbred lines and single-cross hybrid components of four double-cross hybrids reported earlier (Hunt *et al.*, 5). These samples were grown during different years at different locations. Another series of samples involved a group of three inbred lines and crosses which was organized for the purpose of studying heterozygosity or plant vigor. The three lines were crossed as single crosses F_1 and F_2 generations, back crosses, three-way crosses, and a fourth type of cross similar to a double-cross hybrid, in which one of the three lines appeared twice (hereinafter designated as "double cross" for the sake of ease of expression). The third series consisted of early, uniform single-cross hybrids obtained by crossing each of nine inbred lines with the other eight lines. The latter two series of corn were grown at the Ohio Agricultural Experiment Station in 1946. Each sample analyzed was a composite of five replications grown in the same field under similar environmental conditions.

The methods of analysis were the same as those used in previous studies (5).

Results and Discussion

The vitamin values for the double-cross hybrids (5) and the component lines and single crosses which enter into their genetic make-up are given in Table I. These samples, as previously stated, were gathered from different seasons and locations, so that some of the variation in vitamin content might be ascribable to environmental factors, especially in the case of pantothenic acid. The four hybrids are related.

There was no significant difference in the average niacin content of the four double-cross hybrids. Among the inbred lines, Oh02 had the highest niacin content (2.91 mg./100 g.), while Ind.WF9, Ind.38–11, and Ill.R4 assayed 2.16, 2.01, and 1.89 mg., respectively. C.I.187–2 (1.56 mg.), Ill.Hy (1.24 mg.), Ia.L317 (1.23 mg.), and Oh40B (1.15 mg.) assayed low in niacin. The single cross highest in niacin, Oh40B × Oh02, contained the high niacin inbred Oh02. The single cross lowest in niacin (Hy × L317) contained two low-niacin inbreds. The single crosses composed of the intermediate-niacin inbreds give values between the two extremes.

Slight differences in the niacin content of the double-cross hybrids showed that the hybrid highest in niacin, Ohio C38, contained the high-

niacin inbred, Oh02, one intermediate-niacin inbred (Ind.WF9), and two low-niacin inbreds, Ill.Hy and Oh40B. U.S.35, nearly as high as Ohio C38, contained three intermediate inbreds (Ind.WF9, Ind.38–11, Ill.R4), and one low inbred (Ill.Hy). The hybrids U.S.13 and Ill.201 contained two intermediate and two low inbreds.

TABLE I

NIACIN AND PANTOTHENIC ACID CONTENT OF DOUBLE-CROSS HYBRIDS AND COMPONENTS (MG./100 G.—10% MOISTURE BASIS)

	Number of samples	Niacin average and P.E.	Pantothenic acid average and P.E.
Ohio C38 (WF9 × Hy) (Oh40B × Oh02)	13	2.13 ± .04	0.54 ± .02
U.S.35 (WF9 × Ind.38–11) (Ill.R4 × Ill.Hy)	14	2.11 ± .03	0.53 ± .02
U.S.13 (Ill.Hy × Ia.L317) (Ind.WF9 × Ind.38–11)	14	1.95 ± .04	0.49 ± .03
Ill.201 (Ind.WF9 × Ind.38–11) (C.I.187–2 × Ia.L317)	14	2.02 ± .03	0.56 ± .03
Oh02	3	2.91 ± .07	0.61 ± .01
Oh40B	3	1.15 ± .04	0.53 ± .04
Ind.WF9	4	2.16 ± .08	0.74 ± .03
Ind.38–11	5	2.01 ± .09	0.77 ± .06
Ill.R4	1	1.89	0.45
Ill.Hy	6	1.24 ± .04	0.49 ± .07
Ia.L317	4	1.23 ± .03	0.52 ± .04
C.I.187–2	3	1.56 ± .04	0.59 ± .04
Oh40B × Oh02	1	2.92	0.52
Ind.WF9 × Ill.Hy	1	2.12	0.19
Ind.WF9 × Ind.38–11	1	2.01	0.50
Ill.R4 × Ill.Hy	1	2.09	0.53
Ill.Hy × Ia.L317	1	1.38	0.39
C.I.187–2 × Ia.L317	1	1.48	0.53

It appears from these data that the niacin content of both the single-cross and double-cross hybrids is related to the niacin content of their component inbred lines.

The average pantothenic acid assay values for the double-cross hybrids were similar. Inbreds Ind.WF9 and Ind.38–11 averaged

higher in pantothenic acid than the other inbred lines, with C.I.187–2 next in line. The double-cross hybrid highest in pantothenic acid, Ill.201, has these three inbreds in its makeup. However, the pantothenic acid content of the double-cross hybrids did not resemble as closely the pantothenic acid content of their components as was the case with niacin. Previous work (Hunt *et al.*, 5) has shown that pantothenic acid is influenced markedly by environmental factors which may mask some of the effects of hereditary factors.

TABLE II

NIACIN AND PANTOTHENIC ACID CONTENT OF SAMPLES FROM STUDY OF HETEROZYGOSITY (MG./100 G.—10% MOISTURE BASIS)

Type of sample		Niacin	Pantothenic acid
Inbred lines	51A[1]	2.20	0.59
	56A[1]	1.74	0.62
	40B[1]	.95	0.60
Single cross hybrids F_1 generation	51A × 56A	2.10	0.66
	51A × 40B	1.78	0.57
	40B × 56A	1.97	0.73
Same, F_2 generation	51A × 56A	2.01	0.76
	51A × 40B	1.74	0.63
	40B × 56A	1.65	0.68
Back crosses	(51A × 56A) (51A)	2.15	0.66
	(51A × 56A) (56A)	1.95	0.77
	(51A × 40B) (51A)	2.01	0.56
	(51A × 40B) (40B)	1.34	0.60
	(40B × 56A) (40B)	1.49	0.75
	(40B × 56A) (56A)	1.85	0.85
Three-way crosses	(51A × 56A) (40B)	2.06	0.63
	(51A × 40B) (56A)	2.15	0.69
	(40B × 56A) (51A)	2.26	0.59
Double crosses	(51A × 56A) (40B × 56A)	1.88	0.88
	(51A × 40B) (51A × 56A)	1.98	0.52
	(51A × 40B) (40B × 56A)	1.73	0.63

[1] 51A, 56A, and 40B are all Ohio hybrids.

The results of the analyses of the group of lines and various types of crosses organized for the study of heterozygosity are shown in Table II. The three inbred lines Oh51A, Oh56A, and Oh40B differed markedly in niacin content, while the variations in this vitamin are not so pronounced in the hybrids. The single crosses, both F_1 and F_2 generations, the three-way crosses, and the double crosses all had niacin values which appeared more closely related to Oh51A and Oh56A than to Oh40B. The single crosses F_1 generation had slightly higher niacin than the F_2 generation. The back crosses with Oh51A

and Oh56A also were similar to these lines in niacin content, whereas those with Oh40B showed a lowering of niacin, though neither was as low as the inbred itself. With the exception of the three-way cross, in which Oh51A was one parent, the niacin values of the hybrids all fell between the limits established by Oh51A and Oh40B, the highest and lowest inbreds, respectively.

TABLE III

NIACIN AND PANTOTHENIC ACID CONTENT OF SINGLE CROSS CORN HYBRIDS (MG./100 G.—10% MOISTURE BASIS)

Inbred	Ill.A		A153			A158		
Crossed with	Niacin	Panto. acid	Crossed with	Niacin	Panto. acid	Crossed with	Niacin	Panto. acid
A153	1.94	0.54	Ill.A	1.94	0.54	Ill.A	2.21	0.41
A158	2.21	0.41	A158	2.22	0.40	A153	2.22	0.40
WR3	2.16	0.50	WR3	1.90	0.54	WR3	2.66	0.22
W3	2.56	0.38	W3	2.47	0.32	W3	2.66	0.27
W22	2.20	0.46	W22	2.21	0.32	W22	2.22	0.23
Oh51A	2.29	0.45	Oh51A	2.03	0.23	Oh51A	2.61	0.25
B8	2.39	0.41	B8	2.49	0.31	B8	2.59	0.31
B9	1.13	0.43	B9	1.62	0.29	B9	1.89	0.34
Mean	2.11	0.45	Mean	2.11	0.37	Mean	2.38	0.30
Inbred	WR3		W3			W22		
Ill.A	2.16	0.50	Ill.A	2.56	0.38	Ill.A	2.20	0.46
A153	1.90	0.54	A153	2.47	0.32	A153	2.21	0.32
A158	2.66	0.22	A158	2.66	0.27	A158	2.22	0.23
W3	2.09	0.37	WR3	2.09	0.37	WR3	2.38	0.34
W22	2.38	0.34	W22	3.04	0.29	W3	3.04	0.29
Oh51A	2.10	0.41	Oh51A	2.70	0.32	Oh51A	2.46	0.30
B8	2.58	0.32	B8	3.04	0.42	B8	3.11	0.28
B9	1.74	0.36	B9	1.94	0.31	B9	1.99	0.40
Mean	2.20	0.38	Mean	2.56	0.34	Mean	2.45	0.33
Inbred	Oh51A		B8			B9		
Ill.A	2.29	0.45	Ill.A	2.39	0.41	Ill.A	1.13	0.43
A153	2.03	0.23	A153	2.49	0.31	A153	1.62	0.29
A158	2.61	0.25	A158	2.59	0.31	A158	1.89	0.34
WR3	2.10	0.41	WR3	2.58	0.32	WR3	1.74	0.36
W3	2.70	0.32	W3	3.04	0.42	W3	1.94	0.31
W22	2.46	0.30	W22	3.11	0.28	W22	1.99	0.40
B8	2.74	0.41	Oh51A	2.74	0.41	Oh51A	2.02	0.32
B9	2.02	0.32	B9	2.13	0.40	B8	2.13	0.40
Mean	2.37	0.34	Mean	2.63	0.36	Mean	1.81	0.36

The pantothenic acid content of the three inbred lines was nearly the same. The range of pantothenic acid values for the whole study was not as wide as the range of niacin values. Several hybrids yielded higher pantothenic acid than did the inbred lines, and variations were

more pronounced among the hybrids than among the lines. In these respects pantothenic acid differed from niacin. The line Oh56A seemed responsible for highest pantothenic acid, Oh40B intermediate, and Oh51A for lowest pantothenic acid in back crosses, three-way crosses, and double crosses. The highest pantothenic acid value obtained was in the double-cross hybrid in which Oh56A appeared twice; the lowest value was in the double-cross hybrid in which Oh51A appeared twice.

Table III shows the niacin and pantothenic acid content of the early, uniform single-cross hybrids. These data are arranged so that each inbred appears as a common parent crossed with eight other inbred lines. The data were analyzed statistically according to the following procedure: Group comparisons were made for each inbred line with each other inbred line by omitting, in each two groups, the single-cross hybrid containing the two lines which were being compared. For example, in the two groups which compared the effect of lines Ill.A and A153, the single cross Ill.A × A153 was omitted. The results of the statistical analysis are shown in Tables IV and V.

TABLE IV

EFFECT OF INBRED LINES ON NIACIN CONTENT OF SINGLE CROSS HYBRIDS

Line B8 higher than W3, W22, A158, Oh51A, WR3*, Ill.A*, A153**, B9**
Line W3 higher than W22, A158, Oh51A, WR3, Ill.A*, A153*, B9**
Line W22 higher than A158, Oh51A, WR3, Ill.A, A153, B9**
Line A158 higher than Oh51A, WR3, Ill.A, A153, B9**
Line Oh51A higher than WR3, Ill.A, A153, B9**
Line WR3 higher than Ill.A, A153, B9**
Line Ill.A higher than B9** (same as A153)
Line A153 higher than B9**
Line B9 lower than all other lines

* Significant.
** Highly significant.

TABLE V

EFFECT OF INBRED LINES ON PANTOTHENIC ACID CONTENT OF SINGLE CROSS HYBRIDS

Line Ill.A higher than WR3, A153*, B8**, B9**, Oh51A**, W3**, W22**, A158**
Line WR3 higher than A153, B8, B9, Oh51A, W3, W22, A158*
Line A153 higher than B8, B9, Oh51A, W3, W22, A158
Line B8 higher than Oh51A, W3, W22, A158 (same as B9)
Line B9 higher than Oh51A, W3, W22, A158
Line Oh51A higher than W22, A158 (same as W3)
Line W3 higher than W22, A158
Line W22 higher than A158
Line A158 lower than all other lines

* Significant.
** Highly significant.

The line B8 as the common parent produced single-cross hybrids having the highest niacin content. This effect was significant when compared with Ill.A and WR3 and highly significant when compared

with B9 and A153. Hybrids containing B9 assayed lowest in niacin; the difference between B9 and each other line was highly significant. The single cross B8 × W22 contained the highest niacin (3.11 mg./100 g.) and B9 × Ill.A contained the lowest (1.13 mg.).

The line Ill.A as the common parent produced single-cross hybrids having the highest pantothenic acid content. This effect was significant when compared with A153 and highly significant when compared with B8, B9, 51A, W3, W22, and A158. Hybrids containing A158 assayed the lowest in pantothenic acid. The difference between A158 and the other lines was significant with WR3 and highly significant with Ill.A. The single crosses A153 × Ill.A and WR3 × A153 contained 0.54 mg. per 100 g., the highest pantothenic acid value; the lowest value, 0.22 mg. per 100 g., was found in the single cross WR3 × A158.

In the analysis of uniform single-cross hybrids, both niacin and pantothenic acid were influenced definitely by hereditary factors.

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EFFECT OF BENTONITE ON LOAF VOLUME AND WEIGHT OF HARD AND SOFT WHEAT BREAD [1]

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ABSTRACT

The responses of a soft and a hard wheat flour to naturally occurring bentonite (Volclay, B. C. Dust) as it affects loaf volume and weight were investigated. Both the straight dough and sponge and dough processes of making bread were used.

The loaf volume of breads made from a hard wheat flour progressively increased as the amounts of bentonite were increased up to 0.8 or 1.0%. These breads compared favorably in color and texture with bread containing 2 mg. % potassium bromate. On the other hand, loaf volume decreased with increasing amounts of bentonite when a soft whole wheat flour was used in the straight dough process.

The observed increase in loaf volume is not due to any action of the bentonite on the yeast. A bentonite-treated yeast suspension produced a loaf which was smaller than the control.

The weight of the loaf tended to show a slight increase as the content of bentonite increased. This is no doubt due to a slightly higher water content.

Different theories, supported by experimental observation, have been advanced in an effort to understand the beneficial effects of certain oxidizing compounds on the texture and loaf volume of bread. The mechanism by which these flour improvers may act has been explained principally in two different ways, viz., the retardation of proteinase activity inherent in the flour and/or their action on certain reducing groups or bonds in the flour proteins or other dough ingredients. Hildebrand (5) has very ably reviewed the numerous reports in the literature on this subject.

Earlier work by the present writer (4) indicated that the proteolytic activity of papain was retarded in the presence of bentonite (Volclay, B. C. Dust). Free titratable acidity in a gluten-papain suspension was increased 13 times above the initial value during an incubation period of four days compared to an increase of only seven times in the gluten-papain-bentonite suspension. Corresponding increases in the amino acid content (8) were six times for the gluten-papain suspension, four times for the gluten-papain-bentonite suspension. Could this inorganic material act as a proteinase inhibitor and hence a flour improver? The observations of Ensminger and Gieseking (2) de-

[1] Manuscript received January 14, 1944.
Work was supported by American Colloid Company, Chicago, Illinois.
Published as Scientific Paper No. 766, College of Agriculture and Agricultural Experiment Stations, Institute of Agricultural Sciences, State College of Washington, Pullman, Washington.
[2] Now located at the State College of Washington, Pullman, Washington.

scribing the adsorption of proteins by montmorillonitic clays appeared further to justify raising this question.

Observations on the effect of bentonite on the loaf volume and weight of both a hard and a soft wheat bread are reported. In addition the total reduced nitrogen and total solids of the water extract, as well as the swelling capacity of the bread crumb made from a hard whole wheat flour, are given.

Materials and Methods

Breads with varying amounts of bentonite were prepared by both the straight dough and the 50% sponge and dough processes. Recognizing that differences in either the protein content or the proteolytic activity of flours, as well as the method of manipulation of the doughs, might influence any responses to the bentonite treatment, both soft wheat and hard wheat flours were used.

Basic ingredients were:

	Straight dough process	*Sponge and dough process*	
		Sponge	*Dough*
	g.	*g.*	*g.*
Flour	200	100	100
Nonfat milk solids	8	8	—
Shortening (hydrogenated vegetable)	6	—	6
Sodium chloride	3	—	3
Yeast (compressed)	6	6	—
Sucrose	12	8	4
Water—amount for optimum absorption	—	—	75 ml.
Bentonite—variable per cent based on the weight of the flour			
Potassium bromate—2 mg. % based on the weight of the flour (in only those mixes as indicated in the tables)			

Manipulation of Doughs:

1. Straight dough process: The dough was punched every 5 minutes for 20 minutes, given a 15-minute rest period, panned, proofed for another 65 minutes, and baked.
2. Fifty per cent sponge and dough process: The sponge was fermented 3.5 hours at 36.5°C. ± 1.5°, mixed with the dough ingredients, fermented for another 50 minutes, punched, given a 15-minute rest period, panned, proofed for 50 minutes, and baked.

Doughs made from the above ingredients were mixed by a mechanical mixer, divided into halves, proofed at 36.5°C. ± 1.5° in a constant temperature cabinet, and baked in a reel type oven at 232°C. (450°F.) for 23 minutes. One to two hours after removing from the oven, weight and volume of the separate loaves were observed.

Tests on Bread Crumb:

1. Swelling capacity was determined by the technique of Cathcart and Luber (1) with slight modification. Tests were made on the 2-hour-old bread.
2. Total water-soluble nitrogen—determined by the micro-Kjeldahl method on the water extract obtained from the swelling capacity test.
3. Total solids of water-extract—aliquots of the centrifugate from the determination of the swelling capacity were air-dried at 100°C. ± 1° for 6 hours, cooled, and weighed.

Results and Discussion

With increasing increments of bentonite up to 0.8% of the flour used, loaf volume progressively increased with a hard wheat flour (Table I). Even with 0.2% added bentonite a volume greater than

TABLE I

EFFECT OF BENTONITE ON LOAF VOLUME AND WEIGHT OF A HARD WHEAT BREAD [1]

Bentonite (%)	$KBrO_3$ (mg. %)	Straight dough process[2]		Sponge and dough process	
		Weight (g.)	Volume (ml.)	Weight (g.)	Volume (ml.)
—	—	159.0	532	160.8	462
0.2	—	164.2	542	162.8	492
0.4	—	163.5	617	158.5	520
0.6	—	157.2	632	160.5	538
0.8	—	170.0	710	163.2	545
1.0	—	—	—	167.0	540
—	2.0	159.2	655	155.8	495

[1] Average values of duplicate loaves.
[2] Bran removed with 40-mesh sieve.

that for the control was obtained. Loaves were superior in volume to that of the bromated breads (2 mg. % potassium bromate) with 0.6 to 0.8% bentonite using the straight dough process; with 0.2 to 0.4% bentonite using the sponge and dough process. Loaf weight also increased. Furthermore, the crumb color, aroma, and texture of the breads with 0.6 to 1.0% bentonite compared favorably with the bromated breads (Fig. 1). With either method of fermentation the treated breads had thinner cell walls, lighter crumb, and lighter color than the controls (A-2 and B-1) which contained neither bentonite nor bromate. The volumes of the bentonite-treated bread (A-10 and B-11) were even greater than those of the bromated loaves (A-11 and B-13).

The effect of bentonite on the soft whole wheat flour was opposite to that observed with the hard wheat flour: a decrease in loaf volume

with the former, an increase in the latter (Table II). Cell walls of the crumb of the bread made from the soft whole wheat flour were thick, heavy, and of a coarse texture.

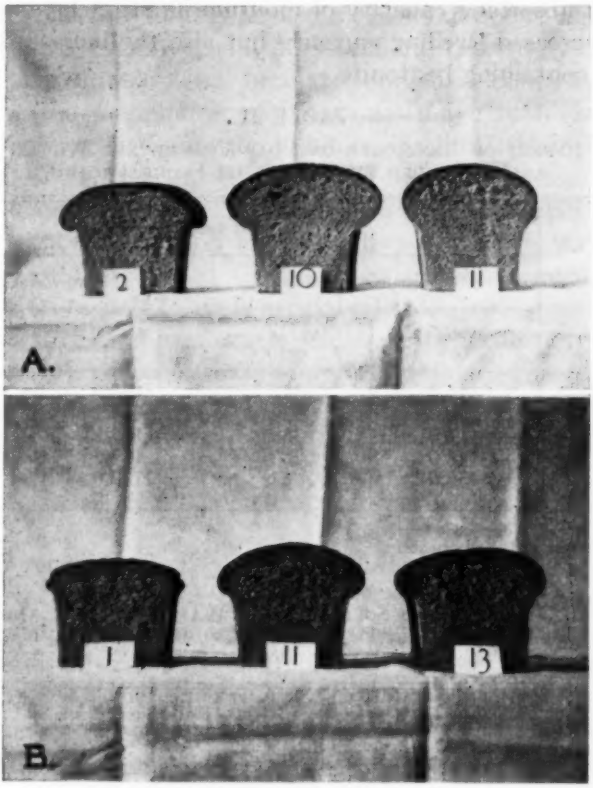

Fig. 1. Effect of bentonite on loaf volume and texture using a hard wheat flour.

A. Straight dough process
Loaf 2—Control, no bentonite or bromate
Loaf 10—0.8% bentonite
Loaf 11—2 mg. % bromate

B. 50% sponge and dough
Loaf 1—Control, no bentonite or bromate
Loaf 11—1.0% bentonite
Loaf 13—2 mg. % bromate

Evidence points toward retarded protein degradation in the bentonite-treated bread (Table III). In freshly baked breads made from a hard whole wheat flour, there was less reduced nitrogen in the water extract of the bentonite-treated breads than in either the bromated or control breads. Likewise, the total solids in the water extract of the bentonite-treated breads were less than either the bromated or control breads.

On the other hand, the swelling capacity of the bentonite-treated bread was higher than that of the others. Doughs to which increasing amounts of bentonite were added required increasing amounts of water to secure an optimum absorption of moisture. No doubt the large water-absorbing capacity of montmorillonitic clays explains not only the increased swelling capacity but also the increased weight of the loaves containing bentonite.

TABLE II

EFFECT OF BENTONITE ON LOAF VOLUME AND WEIGHT AS INFLUENCED BY QUALITY OF FLOUR PROTEIN

Bentonite (%)	Straight dough process[1]			
	Soft whole wheat flour		Hard wheat flour[2]	
	Weight (g.)	Volume (ml.)	Weight (g.)	Volume (ml.)
—	144.0	348	159.0	532
0.2	146.0	322	164.2	542
0.4	147.0	350	163.5	618
0.6	148.0	280	157.2	632
0.8	138.0	262	170.0	710

[1] Average values of duplicate loaves.
[2] Bran removed with 40-mesh sieve.

TABLE III

TOTAL NITROGEN AND SOLIDS OF THE WATER EXTRACT AND THE SWELLING CAPACITY OF BREAD CRUMB MADE FROM A HARD WHOLE WHEAT FLOUR [1]

Treatment	Water-soluble nitrogen (mg. %)	Total solids of water extract[2] (g.)	Swelling capacity[2] (ml.)
Control	225.7	8.8	286.7
$KBrO_3$ 2 mg. %	220.5	8.8	238.0
Bentonite 0.4%	206.5	7.8	292.2
0.8%	181.8	7.6	346.3

[1] Sponge and dough process.
[2] Per 100 g. bread crumb.

However, these observations do not explain the difference in the response of the soft wheat and hard wheat flours to the added bentonite.

To determine whether the favorable action of bentonite was on the yeast or the flour, bread leavened with bentonite-treated yeast was made from hard wheat flour. The yeast and 0.4% bentonite were intimately mixed with an electric mixer.

Following refrigeration at 8°C. ± 2° for 10 hours, the bentonite-yeast suspension was used in making bread by the sponge and dough

process. Yeast for the control and bromated breads was treated in the same manner except that no bentonite was added to the suspension. The loaf volumes were as follows: control loaves, 452 cc.; bromated loaves, 505 cc.; bentonite loaves, 392 cc. That the smaller loaf volume is due to the bentonite treatment of the yeast and not to injury of the yeast cells by the severe mechanical treatment is shown by the normal volumes of the control and bromated breads. Some constituent necessary for the metabolism of yeast may have been irreversibly adsorbed onto the clay during the 10 hours standing in the refrigerator. The work of Ensminger and Gieseking (3) indicates that as a protein molecule is adsorbed in an acid medium as a cation, the base exchange capacity of the bentonite is definitely reduced. In this way the clay may either have inactivated the yeast cells or have been rendered inactive itself in respect to its beneficial effects.

In the slightly acid medium in which fermentation of the bread doughs occurs, it is probable that "powerful but latent" proteinases (6), or some of the flour proteins, are positively charged. As such, and acting as cations, they might be adsorbed onto the negatively charged bentonite. If the labile radical of a proteinase was thus removed from the field of activity, its proteolytic action on the flour proteins would be retarded.

If similar reasoning is applied to the flour proteins they should be less subject to proteolytic attack or their stability increased toward reducing agents inherent in fermenting dough. The work of Olcott, Sapirstein, and Blish (7) would appear to support this last theory. On the other hand, the beneficial effect may result from the adsorption of reducing groups formed during the mixing and fermentation.

However, the foregoing assumptions fail to account for the response of the soft whole wheat flour to bentonite. It is possible that these observations can be explained by a greater degree of solvation of the flour proteins, due to the large water-binding capacity of bentonite. The extent of solvation might have produced an optimum elasticity in the hard wheat flour but was too great for the soft wheat flour. This would result in a weakened flour protein and a smaller loaf of bread for the soft wheat flour.

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A NOTE ON THE EFFECT OF CHLORINE-SUBSTITUTED PHENOXY COMPOUNDS ON THE MILLING AND BAKING QUALITY OF HARD RED SPRING WHEAT [1]

L. D. SIBBITT and R. H. HARRIS [2]

ABSTRACT

The work described shows that application of the acetate, triethyl amine, and butyl ester of 2,4-dichlorophenoxyacetic acid to wheat plants at different stages of growth did not impair milling and baking quality. There was some evidence that the protein content and baking quality were improved by some of the treatments.

Organic substances possessing growth-promoting activity are becoming increasingly important in the field of plant research (2, 3). With the discovery of the selective action of the different compounds emphasis has become concentrated on weed-killing action. Since 1934 more than one thousand derivatives of phenoxy acetic acid and similar substances have been tested as growth regulators or weed killers.

The purpose of this preliminary study was to ascertain if any harmful effects upon milling and baking quality would result from the use of 2,4-dichlorophenoxy compounds on growing wheat. Five varieties of hard red spring wheat were included in the present inquiry. Applications of three 2,4-dichlorophenoxy compounds were made when the plants were at the tiller stage in one experiment. In a second experiment the compounds were added at four different stages of plant growth of one wheat variety only. The wheat was planted on weed-free, hand-cultivated plots, consisting of three 18-foot rows 12 inches apart, and replicated four times. Sixteen feet of the center row of each plot was used for this study. The compounds were added in aqueous solution adjusted to yield one pound of acid equivalent per acre. The wheat harvested from these plots was milled and baked by micro methods (1) and the wheat protein content ascertained (Kjeldahl-Gunning method).

[1] Manuscript received April 19, 1948.
[2] North Dakota Agricultural Experiment Station, Fargo, North Dakota.

The data and tentative conclusions secured from this study are presented in this note.

Table I shows the mean results obtained from the five wheat varieties from the plots treated with the three phenoxy compounds noted. These compounds appeared to affect all the five varieties in

TABLE I

EFFECT OF THREE PHENOXY COMPOUNDS ON MILLING AND BAKING QUALITY

Treatment [1]	Protein content	Flour yield	Loaf volume	Crumb color
	%	%	cc.	
A	14.9	68.8	172	7.1
B	14.8	69.1	173	7.1
C	15.5	68.1	183	7.1
D	15.5	68.3	191	7.2

[1] A = No treatment.
B = Sodium 2,4-dichlorophenoxy acetate.
C = 2,4-dichlorophenoxytriethylamine.
D = Butyl ester of 2,4-dichlorophenoxyacetic acid.

the same manner regarding milling and baking quality; therefore the original data are not shown.

Treatments with 2,4-dichlorophenoxytriethylamine and the butyl ester of 2,4-dichlorophenoxyacetic acid increased the protein content approximately 0.6%; the loaf volume was also slightly increased. The other two criteria of quality were not affected by the treatments. The sodium salt had no effect.

The results from the treatment at different growth stages are shown in Table II. These data are means of results obtained by applications

TABLE II

EFFECT OF STAGE OF APPLICATION OF THREE PHENOXY COMPOUNDS ON MILLING AND BAKING QUALITY

Stage	Protein content	Flour yield	Loaf volume	Crumb color
	%	%	cc.	
Control	15.1	71.3	175	8.0
Tiller	15.7	71.1	188	8.5
Boot	15.9	70.2	148	7.8
Bloom	16.2	70.8	168	8.0
Soft dough	15.3	71.3	157	8.0

of the three compounds listed in Table I with one wheat variety (Mida). Application at the tiller stage increased protein content and loaf volume in a comparable manner with the results shown in Table II; however, in this instance loaf crumb color was improved. Treatment at the bloom stage increased protein content most, but had no effect on loaf volume. Application at the boot stage produced 0.8% more protein than the control, but yielded the lowest loaf volume.

Application of the phenoxy compounds to wheat during the various growth stages had no effect on the mixogram pattern.

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BOOK REVIEW

On the Structure of the Protein Molecule. A Chemical Investigation. By N. Trönsegaard. Second edition. 124 pp. Einar Munkgaard, Copenhagen, Denmark, publisher; G. E. Stechert and Co., 31–37 East Tenth Street, New York City. 1944. Price $4.50.

The author has for some years been convinced that at least the globular proteins possess properties which are not consistent with a structure of long polypeptide chains. He points out that the customary hydrolysis to the constituent amino acids is a drastic treatment; consequently he resorts to a mild reduction with sodium in amyl alcohol as a means of stabilizing the linkages before a mild hydrolysis to obtain the protein fragments. He has identified isoamyl amine and several heterocyclic rings containing nitrogen among the products obtained; certain other fractions have been isolated and analyzed in the form of their platinic chloride salts. Among the proteins studied are wheat gliadin and the albumin, globulin, and globin fractions of horse serum.

This monograph is a summary of his findings over a period of some twenty years. From these he is led to postulate that the most characteristic feature of the structure of proteins consists of heterocyclic subunits of essentially the same elementary composition; these units are linked together by short aliphatic chains. His picture has something in common with the Wrinch cyclol theory and with Abderhalden's diketopiperazine postulate. Trönsegaard's conclusions are not the currently accepted views; nevertheless he has done considerable careful analytical work which must be considered in formulating any theory of protein structure. For this reason the book is recommended to anyone concerned with this still unsettled problem.

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SUGGESTIONS TO AUTHORS

General. From January 1, 1948, an abstract will be printed at the beginning of each paper instead of a summary at the end, references will be numbered to provide the option of citing by number only, and date of receipt, author's connections, etc., will be shown in footnotes. Except on these points, authors will find the last volume of *Cereal Chemistry* a useful guide to acceptable arrangements and styling of papers. "On Writing Scientific Papers for Cereal Chemistry" (*Trans. Am. Assoc. Cereal Chem.* **6**: 1–22. 1948) amplifies the following notes.

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Text. Clarity and conciseness are the prime essentials of a good scientific style. Proper grouping of related information and thoughts within paragraphs, selection of logical sequences for paragraphs and for sentences within paragraphs, and a skillful use of headings and topic sentences are the greatest aids to clarity. Clear phrasing is simplified by writing short sentences, using direct statements and active verbs, and preferring the concrete to the abstract, the specific to the general, and the definite to the vague. Trite circumlocutions and useless modifiers are the main causes of verbosity; they should be removed by repeated editing of drafts.

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Use names, not formulas, for text references to chemical compounds. Use plural verbs with quantities (6.9 g. were). Figures are used before unit abbreviations (3 ml.), and % rather than "per cent" is used following figures. All units are abbreviated and followed by periods, except units of time, which are spelled out. Repeat the degree sign (5°–10° C.). Place 0 before the decimal point for correlation coefficients (r = 0.95). Use * to mark statistics that exceed the 5% level and ** for those that exceed the 1% level; footnotes explaining this convention are no longer required. Type fractions on one line if possible, e.g., A/(B + C). Use lower case for farinograph, mixogram, etc., unless used with a proper name, i.e., Brabender Farinograph. When in doubt about a point that occurs frequently, consult the Style Manual or the Dictionary.

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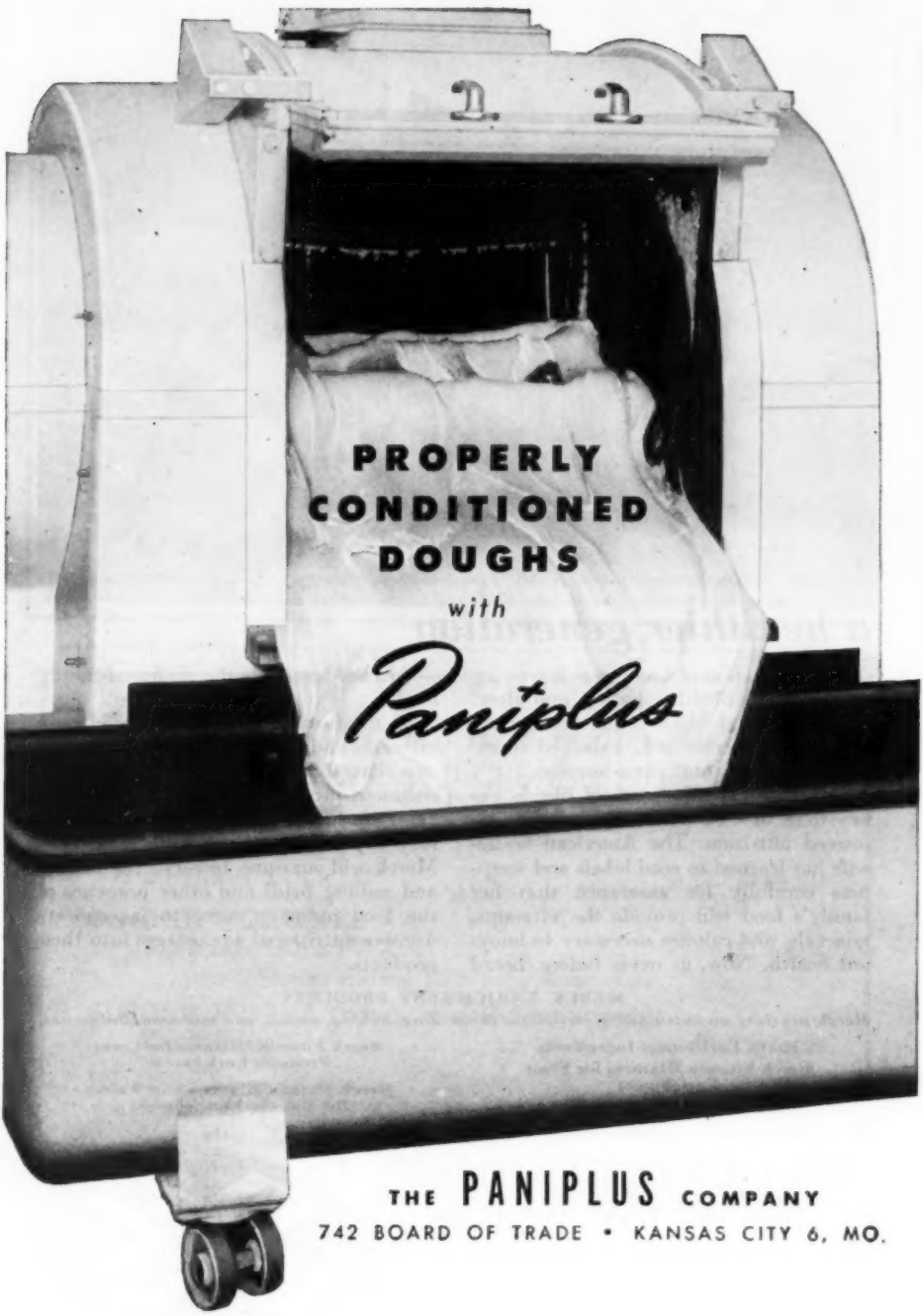

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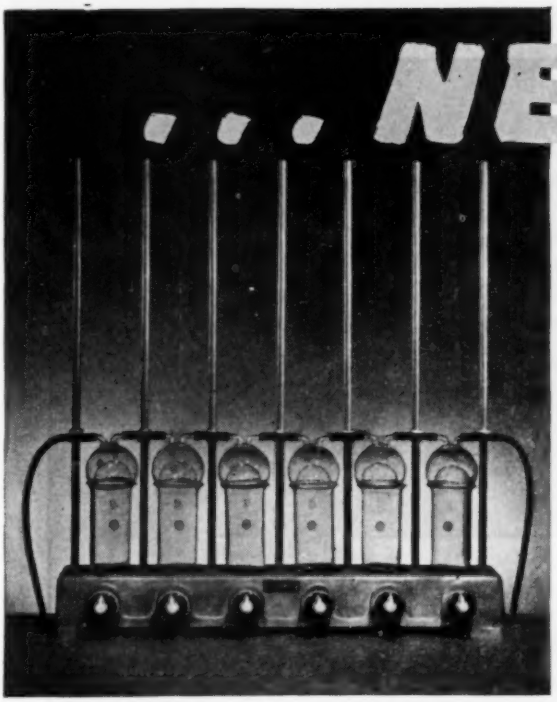

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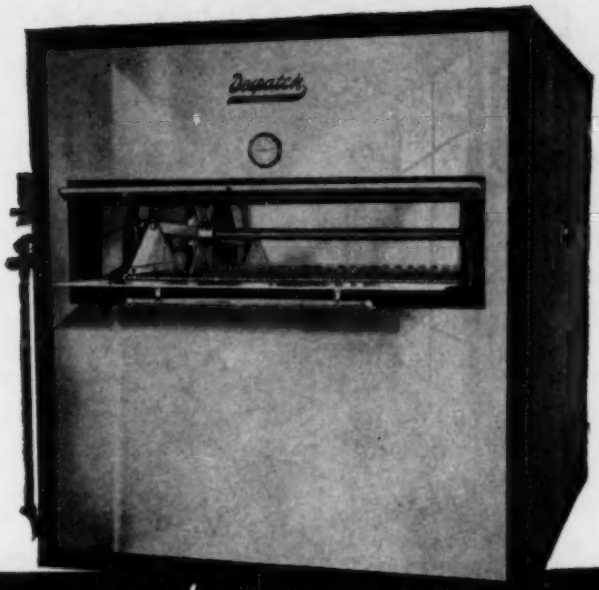

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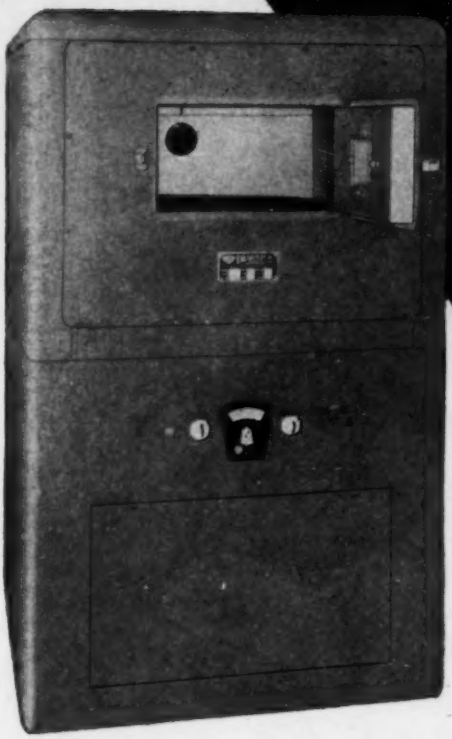

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